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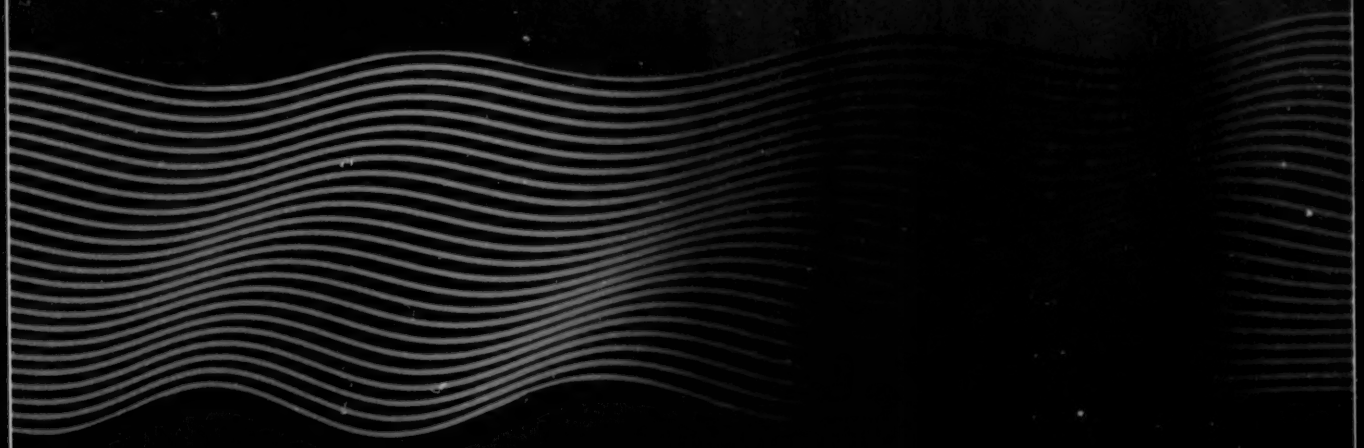

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Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting?[1]

Moira M. Ferguson and Roy G. Danzmann

Abstract: We comment on the role of genetic markers in fisheries and aquaculture with a view to the future. Our goal is to encourage researchers to evaluate the molecular markers they need to deploy and shift their thinking away from analyses of stock structure towards more aggressive pursuit of questions related to genome structure and function. Examples illustrate that no one marker type is appropriate for all applications. Choice should be based on the evolutionary genetic attributes of both the species and the marker loci themselves. We evaluate three relatively new marker types: mitochondrial DNA (mtDNA) sequences, randomly amplified polymorphic DNA, and hypervariable nuclear loci. We conclude that (*i*) sequences of mtDNA do not necessarily detect greater polymorphism than restriction endonuclease analysis, (*ii*) the technical ease of randomly amplified polymorphic DNA is offset by questionable repeatability, and (*iii*) simulations illustrate that even new marker systems with large numbers of alleles need not detect differences among closely related yet significantly differentiated populations. Increasing the number of alleles per locus did not increase the probability of detecting significant differences. Finally, we consider the roles of genetic markers in helping to determine family relationships in pooled lots of fishes and locate genes that control an organism's phenotype (quantitative trait loci). We discuss how knowledge of quantitative trait loci can help us to understand the basis of individual differences in performance.

Résumé : Nous traitons, dans une optique future, du rôle des marqueurs génétiques dans les domaines des pêches et de l'aquaculture. Notre objectif est d'inciter les chercheurs à évaluer les marqueurs moléculaires dont ils ont besoin pour délaisser l'analyse de la structure des stocks en faveur de raisonnements plus proactifs touchant la structure et la fonction des génomes. Des exemples sont donnés pour montrer que l'utilisation d'un seul marqueur ne suffit pas à toutes les applications. Le choix d'un marqueur devrait reposer sur les attributs génétiques évolutifs de l'espèce et du locus du marqueur lui-même. Nous évaluons trois types de marqueurs relativement nouveaux : les séquences d'ADN mitochondrial, l'ADN polymorphe amplifié de façon aléatoire et les locus nucléaires hypervariables. Nous concluons que (*i*) les séquences d'ADNmt ne permettent pas nécessairement de déceler un polymorphisme plus important que l'analyse par endonucléase de restriction, (*ii*) la facilité de la technique de l'ADN polymorphe amplifié de façon aléatoire est annulée par le manque de répétabilité et (*iii*) des simulations montrent que même les nouveaux systèmes de marqueurs à grand nombre d'allèles ne décèlent pas nécessairement les écarts entre des populations apparentées mais significativement différentes. L'accroissement du nombre d'allèles par locus n'accroît pas la probabilité de déceler des écarts significatifs. Enfin, nous examinons le rôle des marqueurs génétiques pour la détermination des rapports familiaux au sein de lots regroupés de poissons et la localisation des gènes qui régissent le phénotype des organismes (locus de trait quantitatif). Nous traitons de la façon dont la connaissance du locus d'un trait quantitatif peut nous aider à comprendre le fondement des différences individuelles du rendement.

[Traduit par la Rédaction]

Introduction

Technological advances in molecular biology and biochemistry have led to the development of a variety of genetic markers that can be used to address questions of relevance to the management and conservation of fish species. Genetic markers have been applied to three fisheries areas in particular — stock structure analysis, aquaculture, and taxonomy/systematics (Ward and Grewe 1994) — with varying degrees of success (Carvalho and Hauser 1994).

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Genetic markers can be categorized based on their transmission and evolutionary dynamics (Park and Moran 1994). Nuclear markers such as allozymes, randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), and variable number of tandem repeat loci (VNTRs: minisatellites, microsatellites) are biparentally inherited. Mitochondrial DNA (mtDNA) markers are maternally inherited and nonrecombining such that they have one quarter the genetic effective population size (N_e) of nuclear markers. This reduced N_e makes mtDNA particularly sensitive to detecting reductions in genetic variation as the result of founder events and population bottlenecks. Furthermore, mtDNA retains a history of past isolations for a longer period relative to nuclear DNA (Billington and Hebert 1991). However, the maternal inheritance of mtDNA limits its ability to provide information on the male component of populations.

The need to detect genetic variation has fueled the development of novel marker systems in fisheries biology. The detection of genetic variation among individuals (i.e., homozygotes and heterozygotes at nuclear loci, different mtDNA

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haplotypes) is a requirement in all applications of genetic markers. Some applications will also require the partitioning of variation among groups of individuals (i.e., groups having different allele or haplotype frequencies). Although some applications (see below) will place greater emphasis on genetic differences among groups (stock structure) (Carvalho and Hauser 1994) and some will focus on differences among individuals within populations (pedigree analysis), the detection of polymorphism remains the key.

We comment on the role of genetic markers in fisheries and aquaculture with a view to the future. Our goal is to encourage researchers to evaluate the molecular markers they need to deploy and shift their thinking away from analyses of stock structure towards questions related to genome structure and function. It is not our intention to evaluate the success of molecular markers in delineating stocks relative to other approaches (such as tagging or meristic variation). Such an evaluation would form the foundation of a perspective in its own right.

We first outline some typical applications of genetic markers from our laboratory and show that no one marker type is appropriate for all applications. We argue that the choice of a genetic marker system should be based on the characteristics of a particular species (interacting with the attributes of the marker type) rather than how recently they have been developed. In fact, a combination of mitochondrial and nuclear markers is the most powerful approach (Ward and Grewe 1994). An evaluation is performed on three relatively new genetic marker systems: mtDNA sequences, RAPD, and hypervariable nuclear loci. Finally, we consider two exciting applications of hypervariable genetic markers. First, hypervariable markers can be used in parentage and pedigree analyses to determine family structure in pooled lots of fishes. Second, the new markers can be used to identify genes that control an organism's appearance, physiology, and life history (quantitative trait loci, QTL). It then becomes possible to determine the genetic bases of inbreeding and variation in individual performance.

Applications of genetic markers

Stock/strain identification

The most common use of genetic markers in fisheries biology is to determine if samples from culture facilities or natural populations are genetically differentiated from each other. The detection of differentiation would imply that the source groups comprise different stocks (Carvalho and Hauser 1994) and should be treated as separate management units (Moritz 1994). Management of natural populations at the subspecific level is based on the idea that stocks have individual patterns of recruitment and mortality and react to harvesting and exploitation independently (Carvalho and Hauser 1994). A common objective of molecular genetic analyses is to find diagnostic differences among presumed stocks in either nuclear allelic types (private alleles) or mtDNA haplotypes. Most often, however, stocks differ in frequencies of the same alleles or haplotypes. As a result, researchers search for significant genetic differentiation among putative stocks and use statistical tests that generally adopt Markov chain (Crow and Kimura 1970) or bootstrapping algorithms (Roff and Bentzen 1989; Zaykin and Pudovkin 1993; Danzmann and Ihssen 1995).

Data from three strains of rainbow trout (*Oncorhynchus mykiss*) being used to develop a synthetic strain for the aquaculture industry in Ontario illustrate the differences in resolution of allozymes, restriction fragment length polymorphisms (RFLPs) in mtDNA, and microsatellites (Table 1). The markers differ in the relative amounts of variation detected within strains (numbers of alleles/haplotypes and observed heterozygosity for nuclear loci). The three types of genetic analyses all indicate that the three strains are genetically heterogeneous; all show significantly different allele frequencies at nuclear loci (microsatellites and allozymes) and frequencies of mtDNA haplotypes. The microsatellite analysis detected the greatest amount of differentiation; the three strains showed highly significant ($P < 0.001$; Markov chain analysis in GENEPOP v.2: Raymond and Rousset 1995) differences in allele frequencies at all six loci. Although certain alleles appeared to be restricted to individual strains (private alleles; data not shown), this appearance may be due to the limited number of individuals analyzed per strain ($N = 42–57$) because additional sampling might detect additional alleles. The mtDNA analysis detected a lower degree of differentiation than the microsatellite analysis. The magnitude of mtDNA frequency differences between strains A and B was relatively small ($P = 0.042$; chi-square Monte-Carlo bootstrapping procedure as used by Danzmann and Ihssen 1995), while strain C showed highly significant differences from the others ($P < 0.001$). The allozyme analysis also detected significant differentiation in allele frequencies among strains (Markov chain analysis) but not at all loci.

We determined the relative amounts of genetic variation partitioned among strains for each marker type (locus or mtDNA haplotype) by calculating *F*-statistics using Weir and Cockerham's (1984) algorithm in GENEPOP (v.3). The F_{ST} value generated from the mtDNA data was much greater than that for any of the nuclear loci (Table 1). The magnitude of this value can be attributed to the existence of haplotypes in strain C that are largely absent in strains A and B. In contrast, most alleles at the nuclear loci were shared among strains (but in different frequencies), accounting for the lower F_{ST} values.

The search for genetic differentiation among rainbow trout strains does not present a particular challenge for genetic markers. Rainbow trout show relatively high levels of genetic variation among individuals relative to many fish species. Furthermore, the different culture histories of the strains would increase the probability that differences would be found among strains (M.M. Ferguson 1994). The power of different marker types is best illustrated by examining population segments of species that have low levels of interindividual variation and are in the process of diverging or have diverged relatively recently.

Thingvallavatn, Iceland (the suffix "vatn" means lake), contains two sympatric morphotypes (benthic and limnetic) of Arctic char (*Salvelinus alpinus*). Each morphotype is composed of two morphs and these differ markedly in ecology, behaviour, and life history (Snorrason et al. 1994). The divergence among the Arctic char morphs is thought to have taken place in the last 10 000 years within the lake. This divergence could have resulted from a combination of reproductive

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Table 1. Comparison of detectable variation at seven allozyme loci, RFLPs in mtDNA, and six microsatellite loci in three strains of rainbow trout.

	Number of alleles/haplotypes			Observed heterozygosity			Population differentiation			
Locus	A	B	C	A	B	C	A vs. B	A vs. C	B vs. C	F_{ST}
Allozyme										
CK-A1	2	1	2	0.55	0	0.31	***	ns	***	0.139
PGM-2	2	2	2	0.35	0.28	0.14	ns	ns	ns	0.003
IDHP-2	2	2	2	0.29	0.36	0.42	**	***	ns	0.087
MDH-B1,2	3	4	4	0.81	0.25	0.75	***	***	***	0.136
bGLUA	2	2	2	0.32	0.56	0.44	***	*	*	0.146
IDHP-3,4	4	4	3	0.71	0.44	0.72	***	ns	***	0.070
SOD	3	2	2	0.56	0.58	0.39	ns	ns	ns	0.016
Average	2.57	2.43	2.43	0.51	0.35	0.45				0.091
N	31	36	36							
mtDNA	1	4	6	—	—	—	*	***	***	0.471
N	24	22	24							
Microsatellite										
Omy2DU	11	13	18	0.46	0.78	0.84	***	***	***	0.062
Omy77DU	5	13	13	0.68	0.66	0.78	***	***	***	0.069
Omy207UoG	9	13	16	0.51	0.56	0.66	***	***	***	0.095
Omy325UoG	10	18	19	0.80	0.63	0.63	***	***	***	0.025
Ssa14DU	5	11	15	0.70	0.55	0.68	***	***	***	0.122
Ssa85DU	14	16	17	0.98	0.79	0.80	***	***	***	0.034
Average	9	14	17	0.69	0.66	0.73	***	***	***	0.068
N	42	45	57							

Note: See text for explanation of tests for population differentiation (ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). *N* is the number of fish analyzed per strain.

isolation among morphs (genetic segregation) and phenotypic plasticity (Skulason and Smith 1995).

We have used a variety of biochemical and molecular marker systems to test whether genetic heterogeneity exists among the Thingvallavatn morphs of Arctic char. Genetic analyses have detected insufficient allozyme (Magnusson and Ferguson 1987), mtDNA (RFLPs, direct sequencing of the control region: Danzmann et al. 1991; Volpe and Ferguson 1996), and single locus minisatellite (Volpe and Ferguson 1996) polymorphism among individuals to test for differentiation. Multilocus minisatellite DNA detected adequate polymorphism but was only able to detect significant differences among morphs belonging to different morphotypes but not within morphotypes (Volpe and Ferguson 1996). Furthermore, the simultaneous detection of polymorphism at many loci limited the amount of information gleaned from the data. Most recently, Gislason (1998) has detected highly significant differences in microsatellite allele frequencies among morphs. Even here, several dozen loci had to be tested to identify those with detectable variation.

Mixed-stock analysis

Many fisheries are composed of mixtures of fish derived from different sources (Utter and Ryman 1993). The differential response of source populations to harvesting makes it important to determine the relative contribution of each to the mixture (Carvalho and Hauser 1994). The estimation of source contributions to the mixed fishery requires the existence of genetic variation among potential source populations. Once such differentiation has been detected, statistical algorithms (e.g., maximum likelihood estimators: Millar 1987) are used to estimate relative contributions of baseline groups (presumed spawning populations) to the mixture.

Most mixed-stock analyses are usually based on either mitochondrial or nuclear markers. However, our experience with walleye (*Stizostedion vitreum*) (McParland 1996) and that of others (e.g., Grewe et al. 1994; Wirgin et al. 1997) suggest that combined analyses with both mitochondrial and nuclear markers might be recommended. For example, spawning populations of walleye from a large geographic area (Tittabawasee River, Saginaw Bay shoals in western Lake Erie) contribute to a commercial fishery in southern Lake Huron (McParland 1996). Particular spawning populations in the Lake Huron – Lake Erie corridor have been identified as especially vulnerable to overharvesting. The combined use of allozyme and mtDNA markers provided the most comprehensive view of population structure in walleye (McParland 1996). For example, there were several cases where one marker was successful in detecting differentiation between a pair of samples while another was not (and vice versa). Furthermore, the different marker types provided different information on the contribution of source populations to the fishery between years.

Fate of cultured fish after stocking

The stocking of cultured fish for the rehabilitation of existing natural populations is a common tool in fishery management. The considerable cost involved is only justified if the stocked fish are surviving and reproducing. Genetic markers can be used to track the success of cultured fish and their descendants if native and cultured stocks differ in the

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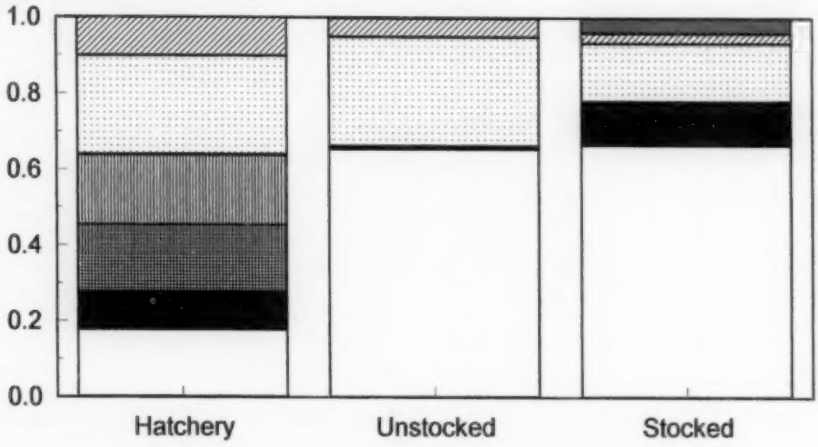
Fig. 1. Comparison of mtDNA haplotype (RFLPs) frequencies in brook trout from Algonquin Provincial Park, Ontario, with those detected in an Ontario hatchery strain. The Algonquin populations (sample sites) have been divided into those that have received hatchery fish and those that have not (data from Danzmann and Ihssen 1995).



frequency of alleles or mtDNA haplotypes. Our recent experience with brook trout (*Salvelinus fontinalis*) in Algonquin Provincial Park, Ontario, suggests that mtDNA markers can provide useful information on the success of cultured fish but they only provide part of the story (Danzmann and Ihssen 1995). Hatchery fish derived from a single strain have been transplanted into the Algonquin Park region since the 1940s and until the early 1980s. The introduced hatchery strain had several mtDNA haplotypes not present in native fish (Fig. 1). Few "hatchery" haplotypes were detected in a survey of over 900 fish from 49 populations, suggesting limited success of hatchery females. Hatchery males could have survived and reproduced (with native fish), but this would not have been detectable because of the maternal inheritance of mtDNA. The level of sampling was adequate for detection of the mtDNA lineages from hatchery fish because the stocking level exerted on many of these wild populations would have been sufficient to replace the entire native populations in a single generation. Interestingly, a parallel study using allozymes suggested survival and reproduction of hatchery fish because large numbers of "hatchery" alleles were detected in Algonquin Park fish (P.E. Ihssen, Ontario Ministry of Natural Resources, personal communication). Thus, an analysis based on either nuclear or mtDNA markers alone would have failed to capture the complexities of the system. The apparent discrepancies between the mtDNA and allozyme analyses can be explained by the differential survival of hatchery males and females and selection on mtDNA haplotypes. The detection of hatchery fish in natural populations is often not possible because many stocks do not show the necessary diagnostic differences (M.M. Ferguson 1994). One solution might be to develop a genetically marked hatchery strain by selective breeding, although such a program runs the risk of the possible introduction of maladjusted genotypes.

Choice of genetic markers

The many marker types available for fisheries and conservation applications (Park and Moran 1994) represent a bewildering array of choices for the uninitiated. The development of new markers has been most necessary for species with little detectable variation among individuals using the old markers. However, relative novelty and not the attributes of the markers themselves has often dictated marker choice. Despite recommendations that common sense should prevail (Utter 1991), early methodologies such as protein electrophoresis are perceived by many as dated and unlikely to attract research funding. The newer generations of genetic markers such as nucleotide sequences of mtDNA, RAPD, and microsatellites have each in turn been touted as panaceas for many applications. This has led in many cases to the abandonment of older marker systems and major retooling of laboratories to use newer systems before the older markers have been applied comprehensively to a particular problem. The constant search for new markers when established ones would suffice has been encouraged by the publication of papers reporting the development of new markers without in-depth application.

It would be very straightforward if a single marker type (e.g., the newer hypervariable microsatellites) were superior for all applications (i.e., if it always detected the greatest amount of population differentiation and provided population specific markers). Laboratories could then concentrate on addressing specific problems instead of succumbing to the pressure to remain "current" and spending resources on new methodology. A valid test to identify the most desirable marker requires that multiple marker systems should be applied to the same species sampled from the same geographic area; most data sets available do not meet this criterion (see examples in O'Connell and Wright 1997). In addition, the sampling effort (numbers of organisms and loci/base pairs examined) should be equivalent among studies based on different markers.

It is apparent that no one marker system is superior for all common applications. For example, Ferguson et al. (1995) reported that mtDNA analysis detected more differentiation in European brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) (as measured by *F*-statistics) compared with allozyme and minisatellite analyses. Microsatellite analysis detected similar differentiation compared with the other nuclear

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markers used (A. Ferguson, Queen's University of Belfast, personal communication). In contrast, O'Connell et al. (1997) detected greater population differentiation among populations of rainbow trout in Lake Ontario with microsatellites than with mtDNA (Dueck and Danzmann 1996). Studies with Atlantic cod (*Gadus morhua*) suggest that microsatellites (Bentzen et al. 1996) and nuclear RFLPs (Pogson et al. 1995) detect greater population differentiation than mtDNA (Carr et al. 1995).

The challenge then becomes to identify attributes that might be used to predict which marker type might be the most useful in a particular context. Ferguson et al. (1995) suggested that attributes of the species (genetic effective population size (contemporary and historical), amount of gene flow (migration)) in combination with those of the marker loci themselves (N_e, selection, mutation rate) can be used to choose an appropriate marker system. For instance, mtDNA is more likely to provide population-specific markers in species with moderate to low N_e (e.g., freshwater salmonids) due to its fourfold greater genetic drift (which results from $\frac{1}{4}N_e$). However, A. Ferguson (1994) warned that the greater susceptibility of mtDNA to drift can lead to spurious conclusions regarding population structuring. This is likely to be a problem in small populations where the haplotype distributions in fish captured during one sampling may not be representative of those in another year.

The relative superiority of mtDNA will be reduced as populations become larger and gene flow increases (marine species). Gene flow will counteract the effects of drift and reduce the likelihood of detecting population differentiation with any marker. Thus, the impact of the difference in N_e between nuclear and mtDNA markers will be negligible in large populations with significant gene flow. The resolution of mtDNA in species with moderate contemporary N_es but have experienced severe historical bottlenecks will depend on the diversity present before the bottlenecks. One possible event leading to bottlenecks is contractions into glacial refugia (Billington and Hebert 1991). mtDNA will not show enhanced resolution in species with little interindividual genetic variation prior to the bottlenecks (i.e., populations will be fixed for the same haplotype). In these cases, it will be necessary to use VNTRs because substantial allozyme variation may also have been lost (usually fewer alleles per locus relative to VNTRs). However, species with greater haplotypic diversity may show a mosaic pattern after the bottleneck due to random lineage extinction (i.e., populations will be fixed for different haplotypes). Ironically, choosing between mitochondrial and nuclear markers is counterproductive given that a combination of both in a single study often provides the most comprehensive view of population structure, possibly because of the different information content of mitochondrial and nuclear markers (e.g., McParland 1996).

Other important factors influencing marker choice are cost and sampling requirements. These aspects have been presented elsewhere (Ferguson et al. 1995) and will not be discussed in detail here. Essentially, the technical simplicity of allozyme analysis is counterbalanced by the need for fresh tissue (the need for internal organs often necessitates lethal sampling). The DNA markers based on polymerase chain reaction (PCR) technology have minimal sampling requirements (nonlethal sampling, archival material), but their implementation is costly and time consuming.

Evaluation of newer marker types

The development of three marker types during the late 1980s – early 1990s generated considerable excitement in the fisheries genetics community. mtDNA sequences, RAPDs, and hypervariable nuclear loci (VNTRs: minisatellites and microsatellites) were welcomed because it seemed possible to test for population differentiation in species with low levels of allozyme variation and mtDNA RFLPs (using the entire molecule). We comment on how these developments have lived up to expectations using a combination of empirical and theoretical data generated in our laboratory.

mtDNA sequences

The ability to sequence PCR-amplified segments of mtDNA led to the perception that direct sequencing has far greater potential to reveal genetic variation within and among populations than restriction enzyme analysis (e.g., Carr and Marshall 1991). Our studies with a variety of fish species indicate that such a conclusion is an oversimplification. The relative ability of each approach to detect polymorphism depends upon the mtDNA regions being sequenced (inherent differences in mutation rates and resultant polymorphism detected) and the number of base pairs being analyzed. Thus, it is not valid to compare the information generated from sequencing a fast-evolving region (e.g., control region) with an RFLP study with low numbers of base pairs analyzed. Sequence analysis of a given region can never be less powerful that restriction enzyme analysis of that same region. Our studies with brook trout (Bernatchez and Danzmann 1993), Arctic char (Volpe and Ferguson 1996), and walleye (Gatt, University of Guelph, unpublished) indicate that sequencing about 300 base pairs from the control region does not detect all of the haplotypic variation as determined from RFLP analysis (using the whole molecule or selected PCR amplified regions). Although sequencing detects greater numbers of haplotypes per base pair analyzed (Bernatchez and Danzmann 1993), our RFLP studies typically sample two to three times the numbers of base pairs accounting for the greater number of haplotypes detected.

RAPD

The presumed attributes of technical simplicity and ubiquitous polymorphism led to the suggestion that RAPDs might have some role in fisheries genetics. Our experience (and that of others) indicates that the dominance/recessive nature of polymorphisms coupled with poor repeatability and inconsistencies makes them very undesirable for most applications. The first step in testing the validity of new markers is to detect polymorphism and establish the inheritance of putative loci. We used 90 random decamer primers to search for RAPD markers in parental rainbow trout and their progeny (unpublished data). Twenty-five of the 90 primers amplified rainbow trout DNA. The average number of intense fragments amplified per primer was 5.69 (range 1–22) of which an average of 2.51 were polymorphic. Despite standardization of protocols, many primers were eliminated from the analysis because amplification patterns were not repeatable and (or) extraparental bands were observed in progeny. Only a small fraction of the presumed loci showed consistent amplification patterns when the same set of individuals was analyzed twice. We focused

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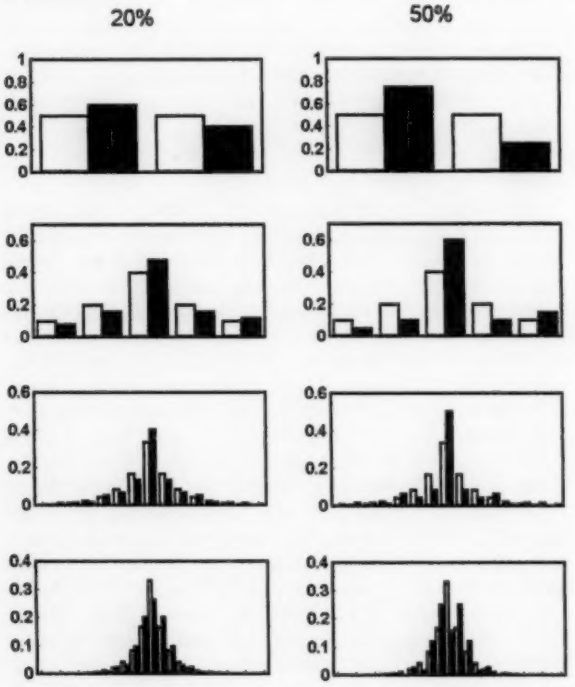
Fig. 2. Allele frequencies at a microsatellite locus with 2, 5, 15, or 25 alleles. The open bars represent the frequencies in a population of 10 000 individuals in Hardy–Weinberg proportions. The solid bars represent allele frequencies in two additional populations that differ from the first (white bars) by 20 and 50%. See text for further explanation.



on 10 polymorphisms where progeny phenotypes segregated according to Mendelian expectations in all families tested. These observations suggest that (*i*) the small number of markers developed is not worth the large amount of effort involved and (*ii*) population-level analyses in the absence of inheritance studies are suspect.

Hypervariable nuclear loci (VNTRs)

One of the greatest challenges in fisheries genetics is to discriminate among closely related populations that share the same alleles but in different frequencies. For example, populations that have only had the opportunity to diverge within the last 10 000 years following glaciation events may be relatively uniform genetically, depending on the amount of variation present when they became isolated. Most species for which there is a concerted effort to search for population differentiation in Canadian waters (e.g., salmonids, Atlantic cod, walleye) fall into this category. The inherently low levels of mtDNA variation in such species (especially in the northern parts of their ranges) have necessitated consideration of more hypervariable markers such as VNTRs. Early indications are that VNTRs (especially microsatellites because they are more widely used) have been successful at detecting population differentiation in species where mtDNA failed to do so (O'Connell and Wright 1997).

We performed a set of simulations to test the effects of allele number and sample size in (*i*) detecting differentiation between two populations that do not differ in allele frequencies (type I error) and (*ii*) failing to detect differentiation between two populations that do differ in allele frequencies (type II error). We were specifically interested in whether or not our ability to detect population differentiation was affected by the number of alleles at a locus (i.e., microsatellites versus allozymes) and what sample sizes would be required for such detection.

To test for type I error, we created a population of 10 000 individuals whose genotypes at a locus (2, 5, 15, or 25 alleles) were in Hardy–Weinberg proportions and where allele frequencies followed a normal distribution (Fig. 2, open bars). This might be representative of a freshwater salmonid population. We then selected a subset of individuals (25, 50, or 100) twice for each simulation with a VBASIC computer program. The two sets of genotypes (from the same starting population) were then analyzed by GENEPOP (v.2) to test for significant differences in allele frequencies. One hundred simulations were run for each combination of allele numbers per locus and sample size (total of 1200). Neither sample size nor allele number affected the probability of type I error (Fig. 3, left panels). The proportion of tests showing significant differences approximated the expected levels of 5% (or less) with the exception of 10% for a sample size of 100 and 15 alleles per locus. These data suggest that the hypervariability of VNTR loci would not increase the probability of falsely detecting differences among populations when such differences do not exist.

In a second set of simulations, we created the genotypic arrays (2, 5, 15, or 25 alleles) for two populations of 10 000 individuals each. The first population showed the same allele frequency distribution as the type I error test (Fig. 2, open bars), while the second differed by 20% (solid bars, left panels) or 50% (solid bars, right panels). Samples of 25, 50, or 100 individuals (genotypes) were sampled from each and significant differences in allele frequencies were tested with GENEPOP (100 simulations per combination, total of 2400). As expected, increasing both sample size and amount of differentiation between populations increased the probability of detecting differences in allele frequencies (Fig. 3, middle and right panels). However, the probability of type II error was high for all sample sizes at 20% differentiation. At 50% differentiation, the probability of type II error was greatly reduced, but only when a sample size of 50 was taken. This suggests that population samplings should be no lower than 50. Similar conclusions were reached by Ruzzante (1998) for estimating genetic distances between populations. Below a sample size of 50, distance measurements were generally inflated, as were estimates of variance partitioning.

The number of alleles per locus did affect the probability of type II error but not in the predicted direction. The least amount of type II error was detected with two alleles for five out of the six combinations of differentiation level and sample size (Fig. 3). The distribution of the number of significant and nonsignificant tests was heterogeneous ($P < 0.05$; 2×4 heterogeneity G-tests) for different allele numbers in four cases ($N = 25/20\%$, $N = 100/20\%$, $N = 25/50\%$, $N = 50/50\%$).

These simulations suggest that marker systems with large numbers of alleles (e.g., microsatellites) do not meet our greatest challenge of detecting significant differences between

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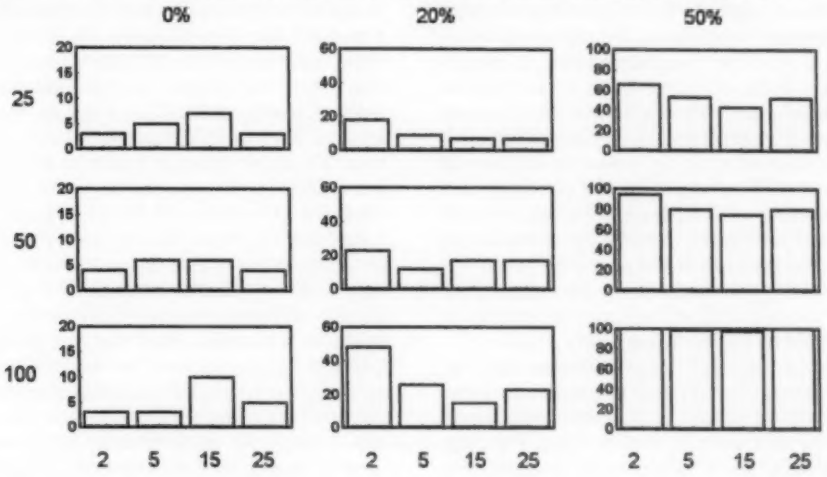
Fig. 3. Number of significant differences in allele frequencies at a microsatellite locus (2, 5, 15, or 25 alleles) between two populations (25, 50, or 100 individuals sampled from 10 000). In one set of simulations the two samples were drawn from the same population (left panels) and in the other two the populations differed by 20% (middle panels) or 50% (right panels) in allele frequencies (see Fig. 2).



genetically similar populations (i.e., 20% difference in allele frequencies). Significant amounts of type II error were detected even with sample sizes of 50–100 individuals. These sample sizes are higher than those typically used (O'Connell and Wright 1997). Corrections made to probability values to protect against type I error (e.g., Bonferroni adjustment) would make it even more difficult to detect heterogeneity among closely related yet significantly differentiated populations. One course of action is to increase sample sizes to ensure that existing differences are detected. Although this will make microsatellite studies even more cost prohibitive, it is necessary because failure to detect differentiation might lead to the management of multiple stocks as a single unit. One controversial approach might be to reconsider the suitability of Bonferroni adjustment.

Interestingly, there seemed to be no advantage in using a locus with more alleles. Given the lower probability of type II error with two alleles (Fig. 3) and the difficulties in scoring hypervariable loci (O'Connell and Wright 1997), it might be advisable to use loci with fewer alleles. This recommendation is especially ironic given that the large number of alleles is one of the most attractive features of these markers. However, it should be noted that higher allele number may be essential when population-specific alleles are desired (with the condition of adequate sampling, see above) and in other applications (see below).

The simulations suggest that microsatellite approaches are expected to be no more powerful than allozyme analyses in studies of population structure, providing that variation is present (i.e., a five-allele system is no better or worse than a 25-allele system). This appears to contradict empirical data (e.g., Table 1) that suggest that microsatellite approaches do seem to be more powerful; the average microsatellite locus had a greater probability of detecting differentiation than the average allozyme locus. One reason for the discrepancy is that the distributions of microsatellite allele frequencies used in the simulations may not be representative of those observed in the empirical data. We specifically simulated the most challenging situation for genetic markers where populations are sharing the same alleles but in different frequency. In contrast, the rainbow trout strains show much greater divergence and do not share all of the same alleles. Thus, the increased propensity for microsatellites to have population-specific alleles may account for the increased resolution in the empirical data. A second reason is that microsatellite analyses may have more apparent power if allozyme allele frequencies are being held constant by selection and microsatellite allele frequencies are drifting stochastically (neutrally) (Ward and Grewe 1994).

The future: beyond analysis of genetic population structure

Although the relative power of microsatellites in detecting population structure has yet to be determined, their enhanced resolution for parentage/pedigree analysis and understanding the genetic basis of individual variation in performance is clear (O'Connell and Wright 1997). It is not possible to conduct these analyses adequately with older marker systems because of their lower levels of polymorphism (allozymes) and type of inheritance (mtDNA, RAPDs).

Parentage and pedigree analysis

The optimal utilization of fish stocks under domestication often requires knowledge of family relationships (parents, sibships) (e.g., Herbinger et al. 1996). For example, in aquaculture breeding programs, the progeny of many males and females are often reared together to minimize environmental variation (confounded with genetic effects). Such conditions are often imposed because of limited rearing space in small commercial facilities. Here, knowledge of family structure can lead to a higher rate of genetic improvement because it becomes possible to identify the progeny of parents with desirable or undesirable characteristics (Doyle and Herbinger 1994). Furthermore, matings between closely related individuals can be avoided. Genetic identification can also increase

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selection intensity for sex-limited traits (spawning date of females, precocial male maturation).

We are performing a pedigree analysis on rainbow trout from a commercial farm to provide useable information for an ongoing breeding program. Specifically, the pedigree information is being used to calculate breeding values for length, weight, and condition factor. Breeding value is the expected progeny performance of a given parental individual relative to the population mean. To expedite the pedigree analysis, we have developed a semiautomated genotyping system where 36 fish can be genotyped at 14 microsatellite loci in a single day (A.G. Fishback, University of Guelph, unpublished). The molecular analysis involves two multiplexed PCR reactions (eight and six loci) whose products are scored in a single extended gel run (ABI Prism 377). Once potential parents and progeny are genotyped, progeny are assigned back to their parents using the program PROBMAX (Danzmann 1997).

The ability to assign progeny back to individual parental pairs depends upon the number of parents contributing to the population mixture and the variability (number of alleles and their frequency) of the microsatellite markers used. We use a second program, PROBMAXG (Danzmann 1997), that allows us to create progeny genotypes from the genotypic combinations detected in the parents. This allows us to assess whether a specific set of microsatellite loci will permit accurate assignment of progeny back to individual parental pairs. Possible progeny genotypic combinations can be simulated when the mating structure is known. However, even when the mating structure is not known but the genotypes of parents contributing to the progeny mixture are known, the possible progeny genotypes arising from all pairwise parental combinations may still be generated and tested. In this case, many more possible progeny/parental matches may result, necessitating the use of additional microsatellite markers for complete discrimination. On average, the hatchery strain we are studying has six alleles at each locus assayed in the multiplexing PCR. This has resulted in over 90% assignment of progeny back to single parental pairs within the largest lot genotyped (135 possible families).

Our analysis is relatively straightforward in that the mating structure is known (potential parents are known). Other researchers use allele-sharing information among progeny to infer genetic relatedness when parental genotypes are unknown (Blouin et al. 1996). These analyses may also incorporate information from population allele frequencies to obtain probability estimates of relatedness (Queller and Goodnight 1989).

QTL

Our perception is that the vast majority of fish geneticists are focused on using genetic markers to search for population differentiation. Although this is understandable given the need to know stock structure for management and conservation (and given current funding opportunities), we believe this focus to be shortsighted in view of the potential of the new hypervariable markers to increase our understanding of the fish genome (M.M. Ferguson 1994). Most traits of relevance to fish biologists (e.g., recruitment, growth) are controlled by many loci that are inherited quantitatively (QTL). Such loci are of considerable economic and evolutionary significance because they are the basis of adaptation and economic productivity. For instance, QTL analysis can be used to demonstrate that a portion of the phenotypic variation among individuals, often ascribed to experimental error by many biologists, is real and under genetic control.

Unknown, polymorphic QTL can only be located using physically linked polymorphic marker loci (Tanksley 1993). Thus, a first step in identifying QTL is to develop a genetic map where the relative chromosomal locations of different types of genetic markers are determined. These markers then serve as "landmarks" for genes that affect a given quantitative trait. We are developing a genetic map in rainbow trout to locate QTL for growth, spawning date, and upper temperature tolerance (Danzmann et al. 1998; Jackson et al. 1998; Sakamoto et al. 1998). We are using five backcrosses that were produced by crossing strains divergent for several quantitative traits. The use of multiple families and different genetic backgrounds will provide unique information on the variability in linkage relationships. Most mapping projects are confined to single pedigrees and this may lead to an unrealistic view of genome dynamics. Although the structure of the map (i.e., order of loci) is likely to be correct, we have observed significant variation in recombination frequencies (i.e., map distances) among families. Our mapping effort is focused on microsatellites because of their hypervariability, relatively uniform distribution throughout the genome (Dietrich et al. 1992), and the ability to use primer sets developed for one species on other closely related species. We first concentrated on RAPD markers but found that the inability to distinguish between heterozygotes and homozygotes (dominant phenotype) in the parents limited the number of locus pairs that could be tested for linkage in the progeny.

AFLPs (Vos et al. 1995) are another type of genetic marker in vogue for gene mapping. The appeal of AFLPs is that a genetic map can be constructed within a relatively short period of time (months). Indeed, a rainbow trout linkage map is now available that is composed of approximately 500 markers, with the majority of markers being of the AFLP type (Young et al. 1998). Interestingly, the AFLP markers appeared to be clustered more tightly around centromeres than other classes of markers used. Also, many of the AFLP markers were tightly linked (three or more markers mapped to one site), with over half (258 markers) clustered into 37 chromosomal sites. The power of AFLPs for QTL mapping will be compromised if these markers are not distributed evenly throughout the genome. The generality of these findings must await further analyses with other fish species.

One potential drawback to AFLP markers for QTL mapping and pedigree analysis is that many appear to be dominantly expressed. The lack of codominant allelic expression for these regions will necessitate densitometric PCR quantitation methods to discriminate between homozygous and heterozygous genotypes. If this can be done reliably, such markers hold great promise for pedigree analyses, as multiple tightly linked AFLP markers could be scored in binary matrix format to facilitate the identification of multiple chromosome arms. Therefore, even if only two allelic states are detectable at the majority of such markers, the detection of a heterozygous state across several markers in a tightly linked cluster (i.e., very low or no recombination) will make it possible to trace the segregation of a given chromosome arm in descendants. For example, if a cluster of 10 markers is mapped to the same chromosomal location, and their genotypes are 1 2 2 1 2

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2 2 0 1 2, where 1 indicates a homozygous dominant genotype, 2 indicates a heterozygous state, and 0 indicates a homozygous recessive state, there are 2^6 possible allelic configurations for the six heterozygous markers in the cluster. Only two of these possibilities will be present, barring recombination. Given this suite of markers, it should then be feasible to identify which individual chromosomal arm was inherited in the progeny. For example, if the above individual (1 2 2 1 2 2 2 0 1 2) is mated to another individual with genotype 2 1 1 1 1 2 1 1 1 1 and genotypes 1 1 2 1 1 2 1 2 1 2 : 1 2 1 1 2 1 2 2 1 1 : 2 1 2 1 1 0 1 2 1 2 : 2 2 1 1 2 2 2 2 1 1 are observed in the progeny, then the two chromosomal arms in the first parent have the following genotypic vector: 1 1 0 1 1 0 1 0 1 0 and 1 0 1 1 0 1 0 0 1 1, where a 0 indicates absence of the AFLP fragment and a 1 indicates amplification of the AFLP fragment. Similarly, the chromosomal genotypes of the other parent would be 1 1 1 1 1 1 1 1 1 1 and 0 1 1 1 1 0 1 1 1 1. Needless to say, such analyses will be highly error prone unless homozygous dominant and heterozygous genotypes can be unambiguously discriminated. For species with a tetraploid ancestry, such as salmonids, the ability to differentiate between four dosage levels (to infer genotypes) may be especially challenging.

Our group has mapped over 150 loci (allozyme, RAPD, mostly microsatellite) and identified 25 linkage groups in rainbow trout. We have also located several chromosomal regions with strong associations and a number of others with weak associations with upper temperature tolerance (Danzmann et al. 1998; Jackson et al. 1998). We are using backcross families where an F_1 male derived between two lines selected for high and low temperature tolerance was backcrossed with females from either line. We have also detected several chromosomal regions associated with spawning date (Sakamoto et al. 1998) and growth (unpublished). Here, daughters spawning on different dates within the spawning season and derived from a backcross mating between a fall-spawning female and an F_1 male between spring- and fall-spawning lines had significant differences in allelic complements at several microsatellite markers. These differences were detectable in two successive years of spawning. The fall- and spring-spawning strains also differed significantly in growth, so it was possible to detect several growth QTL in the same backcross families (unpublished data). These are some of the first QTL to be mapped in a fish species.

The identification of molecular markers tightly linked to QTL will allow the direct determination of how different QTL affect fitness traits (i.e., numbers of loci and type of gene action). A set of models (Cheverud and Routman 1995) have been devised that use genotypic scorings (based on microsatellite markers) and performance values for individuals to determine how QTL affect quantitative traits. These models of physiological epistasis make it possible to determine whether QTL are interacting independently and additively or interact (epistasis) to produce the phenotype. Using a modification of these models, we have found evidence for epistasis for upper temperature tolerance QTL in rainbow trout (Danzmann et al. 1998). Coupled with a pedigree analysis, we also ascertained that paternal alleles originally derived from one strain (high or low temperature tolerance) performed better when backcrossed to females of the same strain. These findings illustrate a form of outbreeding depression in allelic performance.

Once a particular QTL has been mapped to a chromosomal location, it is desirable to determine whether a particular candidate gene is responsible for the phenotypic effect. For instance, we are interested in determining whether any of the temperature tolerance QTL (Danzmann et al. 1998; Jackson et al. 1998) correspond to any of the heat shock protein loci. Once candidate genes are mapped, molecular markers can be used to track allelic variants that may result from structural gene rearrangements related to functional differences or possible cis-regulatory differences in gene expression. These types of changes are difficult (i.e., time consuming and expensive) to characterize in a large number of individuals whereas the use of molecular markers provides a relatively efficient approach for scoring QTL allelic variants in pedigreed family lines.

Molecular markers, QTL, and selective breeding programs

Knowledge of linkage associations between marker loci and QTL can be integrated into selective breeding programs based on biometrical methods. Such an approach, termed marker-assisted selection (MAS), is expected to increase genetic response by affecting intensity and accuracy of selection (Bentsen and Gjerde 1994). Here, fish with a particular genotype/QTL combination associated with a desirable phenotypic effect are used for breeding.

Our data on temperature tolerance QTL (Danzmann et al. 1998) suggest that the integration of marker information into selective breeding programs must proceed with caution. The association between particular microsatellite alleles and the phenotype differs between families. For example, progeny inheriting either paternal QTL chromosomal segment at each of six upper temperature tolerance QTL–marker regions showed differential thermal resistance, and this depended upon the genomic background in which the alleles were expressed (Danzmann et al. 1998). A marker allele associated with a QTL leading to enhanced thermal resistance in one background (either high or low temperature) showed the opposite association in the other genetic background. Although associations between marker alleles and performance traits might be expected to vary between families because of recombination and segregation, such uncoupling is not necessarily expected within a single individual (i.e., the male parent used in our crosses). These results suggest that the effectiveness of MAS may be limited without knowledge of the performance of a given QTL allele in different genomic backgrounds.

Inbreeding depression

Hypervariable genetic markers have other important applications even in the absence of associations with QTL. For example, genetic markers can be used to locate the specific chromosomal regions responsible for inbreeding depression. This would be most feasible with cultured species where parents and their progeny can be managed and traced within a closed system. Classically, variables such as inbreeding coefficients and coefficients of genetic relatedness (r) among individuals have been based on genome-wide estimates of average allele sharing among full sibs ($r = 0.5$), half-sibs ($r = 0.25$), and other associated levels of relationship. It will be possible to use mapped genetic markers to trace the inheritance of specific chromosomal arms in progeny. As an example, in a mating between first cousins, there is 0–25% probability that progeny will inherit an allele identical by descent dependent

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upon the genotypes present in the full-sib parents of the first cousins. These parents have a 25% probability of either possessing identical alleles or not sharing any alleles at a locus or a 50% probability of sharing one allele at the locus. On average, this would result in an inbreeding coefficient of approximately 0.03 (i.e., 0.5^5) for any given allele descended from a grandparent (or 0.06 per locus, since the same probability exists for an allele being identical by descent from either the grandmother or grandfather). Each specific chromosomal region, however, will either have a value of 0 or 1 for the identity by descent for a pair of alleles at that locus. Thus, while most chromosomal regions will not be identical by descent, specific homozygous pairings of identical alleles can be identified in the progeny using molecular markers. The lack of expected homozygous classes may identify chromosomal regions where inbreeding effects are pronounced, leading to lethality.

Final comments

The recent innovations in molecular technology have increased the potential for molecular markers to provide useful information for fisheries management and aquaculture. New molecular markers such as microsatellites appear to have provided the increased resolution to answer questions of stock structure in species with relatively low levels of intraspecific genetic variation. Most significantly, however, the new markers have encouraged new applications such as the determination of family structure in lots of fishes produced from many males and females and the location of genes that control an organism's appearance and understanding how they exert their effects. Researchers should take advantage of these opportunities rather than continue to focus on the more traditional questions relating to genetic population structure.

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Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*)

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Abstract: We examined the effects of four levels of chronic hypoxic stress at three temperatures on the survivorship of *Dreissena polymorpha* and *Corbicula fluminea* to assess the efficacy of O_2 deprivation as a macrofouling control treatment and examine if critical hypoxia limits support reported distribution patterns. At 25°C, the hypoxia tolerance was examined at Po_2 = 7.9, 11.9, 15.9, 23.8, and 31.8 Torr (1 Torr = 133.322 Pa) or 5, 7.5, 10, 15, and 20% of full air O_2 saturation (Po_2 = 159 Torr). At 15°C, the hypoxia tolerance to 7.9, 11.9, and 15.9 Torr was tested and at 7.9 Torr for 5°C treatments. For both species, Po_2 and temperature influenced survivorship dramatically with increasing survivorship at higher Po_2 and decreasing temperatures. At 25°C, *C. fluminea* experienced mortality at 7.9, 11.9, and 15.9 Torr, with LT_{50} values of 144, 216, and 216 h, respectively, versus 288, 384, and 480 h for the 15°C exposures. *Dreissena polymorpha* treatments had LT_{50} values of 120, 216, and 216 h at 25°C for the 7.9-, 11.9-, and 15.9-Torr treatments versus 26% mortality after 600 h and 28% mortality after 720 h at 15°C. The 7.9-Torr treatments at 5°C had LT_{50} values of 480 h for *C. fluminea* and 1056 h for *D. polymorpha*. This study showed that both species displayed broad seasonal variation in hypoxia tolerance and that hypoxia limits may be used to assess infestation risk.

Résumé : Nous avons étudié les effets de quatre degrés d'intensité d'hypoxie chronique, à trois températures différentes, sur la survie de *Dreissena polymorpha* et de *Corbicula fluminea* : il s'agissait d'évaluer l'efficacité de la désoxygénation comme traitement anti-salissures et de déterminer si les limites de tolérance à l'hypoxie sont compatibles avec les profils de distribution observés. À 25°C, nous avons étudié la tolérance à l'hypoxie à des Po_2 de 7,9, 11,9, 15,9 23,8 et 31,8 Torr (1 Torr = 133.322 Pa), ou à des valeurs de saturation de l'air en O_2 de 5, 7,5, 10, 15 et 20% (Po_2 = 159 Torr). À 15°C, nous avons évalué la tolérance à l'hypoxie à 7,9, 11,9 et 15,9 Torr, et à 5°C, nous l'avons évaluée à 7,9 Torr. La Po_2 et la température ont nettement influé sur la survie des deux espèces, le taux de survie augmentant plus la Po_2 était élevée et plus la température était basse. À 25°C, on a observé de la mortalité chez *C. fluminea* à 7,9, 11,9 et 15,9 Torr, avec des valeurs de TL_{50} évaluées respectivement à 144, 216 et 216 h, contre 288, 384 et 480 h lorsque la température était de 15°C. Pour *D. polymorpha*, les valeurs de TL_{50} ont été évaluées respectivement à 120, 216 et 216 h à 25°C aux Po_2 de 7,9, 11,9 et 15,9 Torr, par comparaison à 600 h, pour une mortalité de 26%, et 720 h, pour une mortalité de 28%, à 15°C. À 5°C et une Po_2 de 7,9 Torr, la TL_{50} était de 480 h pour *C. fluminea* et de 1 056 h pour *D. polymorpha*. Ces résultats montrent que la tolérance à l'hypoxie présente une importante variation saisonnière chez les deux espèces étudiées, et que les limites de tolérance à l'hypoxie peuvent servir à évaluer le risque d'infestation.

[Traduit par la Rédaction]

Introduction

The ability of freshwater mussels, native and introduced, to survive short-term exposure ($t \leq 5$ days) to anoxic or hypoxic waters in their natural aquatic habitats has been well documented (Lewis 1984; Holopainen 1987; Sheldon and Walker 1989). However, relatively few studies have examined survivorship of prolonged hypoxic exposure ($t > 5$ days) in freshwater molluscs, focusing instead on the short-term effects of progressive hypoxia on O_2 consumption in unionids (Sheldon and Walker 1989; Massabuau et al. 1991). Prolonged hypoxia exposure tests are rare for marine molluscs as well, with studies limited to the intertidal neogastropod *Stramonita haemastoma* (Kapper and Stickle 1987; Stickle et al. 1989; Das and Stickle 1993) and the marine bivalve *Crassostrea virginica* (Stickle et al. 1989). However, exposure to hypoxic rather than anoxic conditions is more likely to occur in natural aquatic habitats, where ambient O_2 levels usually remain above 2 mg $O_2 \cdot L^{-1}$ (Holopainen 1987; Kapper and Stickle 1987). Additionally, ambient water temperature influences the O_2 demands of most freshwater and marine bivalves (Kapper and Stickle 1987; Sprung 1995). Freshwater bivalve O_2 consumption rates are directly related to ambient water temperatures, with summer O_2 consumption rates being 2.5–30 times the

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winter rates, depending on the species (Hornback et al. 1983; Burky 1983). Finally, some bivalves can demonstrate temperature conditional O_2-dependent and O_2-independent control of their metabolic rate and readily survive short-term periods of anoxic and hypoxic stress (Burkey 1983).

In this study, we attempt to examine the ability of several macrofouling species to survive long-term hypoxic exposure at several different temperatures. Zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*) are important macrofouling species that have caused billions of dollars in damage to industrial facilities in the United States and Canada (Isom 1986; Roberts 1990). Oxygen deprivation is often cited as a potential means of nonchemical control of these molluscs (Claudi and Mackie 1994). This control strategy is based on the poor O_2 uptake regulation of both species under progressively hypoxic conditions (McMahon 1979; Sprung 1995) and their absence in habitats that periodically undergo prolonged anoxia–hypoxia events (Belanger 1991; Dermott and Munwar 1993). Although both observations indicate a low tolerance to prolonged hypoxic exposure, specific long-term hypoxia survivorship data are lacking for both species. It is not known what O_2 levels are critical, the duration endured, or if O_2 levels or durations change with temperature or seasonal condition. By defining the specific O_2 requirements of these species at different seasonal conditions, we address minimum O_2 requirements and determine the critical O_2 levels across a temperature range, the efficacy of O_2 deprivation as a control treatment, and seasonal condition effects on survivorship and, finally, can critical O_2 levels be used to predict what habitats not yet infested are at risk.

Methods

Specimens of *D. polymorpha* were collected from the U.S. Army Corps of Engineers Black Rock Navigation Lock on the Niagara River in Buffalo, N.Y., and transported to Arlington, TX. Immediately after arrival, mussels were washed free of silt with filtered, dechlorinated water and stored in an 800-L aerated holding tank at 5 or 15°C. *Corbicula fluminea* was collected from a small discharge channel at the U.S. Army Corps of Engineers Lake Lewisville Aquatic Plant Research Facility in Lewisville, TX. Asian clams were transported to the laboratory and placed into a 284-L holding tank at 20°C. Hypoxic exposures were initiated on bivalves within 40 days of collection. Bivalves were stored in filtered dechlorinated city of Arlington tap water (pH = 7.8–8.2; Ca^{2+} = 0.18–0.24 mM; total hardness = 118–146 mg·L^{-1}). Bivalves were not fed and were held in complete darkness during acclimation and exposure periods. Borcherding (1992) demonstrated diel variation in the ventilatory behavior of *D. polymorpha* under low food concentrations. We attempted to control this variability by keeping animals under constant darkness for the duration of acclimation and exposure periods. Different temperature exposures were coordinated seasonally, with 25 and 15°C treatments conducted on bivalves collected in spring through fall and 5°C treatments on winter-collected animals.

Thirty clams or mussels were placed into separate 9-cm-diameter glass crystallization dishes, with six replicate dishes prepared for each species (n = 180). Following a 14-day test temperature acclimation period, the dishes were transferred into 4-L plastic aquaria with removable, tightly fitting vented lids. The six aquaria were filled with 3 L of water, leaving a 1-L head space. The aquaria were then placed into an environmental chamber held at the appropriate experimental temperature (25, 15, or 5 ± 0.5°C) and the hypoxic exposure treatments initiated. Three control aquaria were continuously aerated with atmospheric air (P_{O_2} ≈ 159 Torr (1 Torr = 133.322 Pa)), while the other three were held at one of the five tested levels of hypoxia (i.e., P_{O_2} = 7.9, 11.9, 15.9, 23.8, or 31.8 Torr). The criteria for selecting experimental P_{O_2} levels were based on the following: (*i*) establishing large-enough differences in P_{O_2} to reliably determine if O_2 regulatory capacity was occurring and, if so, at what level, (*ii*) differences would not be too large so that when a lower P_{O_2} limit was reached, it could be determined with some degree of precision, and (*iii*) levels should be set at units convenient for control applications (i.e., 5, 7.5, 10, 15, or 20% saturation). The animals used in these experiments had a wide size range, 13.4–33.2 mm shell length for *D. polymorpha* and 13.6–25.7 mm shell length for *C. fluminea*, reflecting the size range of adults in the natural populations.

Oxygen concentrations in hypoxic treatments were maintained with a Cameron GP-5 gas-mixing flowmeter supplied with commercially compressed N_2, O_2, and CO_2. Experimental P_{O_2} concentrations were produced by bubbling water in the treatment aquaria with ratios of N_2 and O_2 that resulted in the maintenance of P_{O_2} levels of 31.8, 23.8, 15.9, 11.9, and 7.9 Torr, while CO_2 concentration was held constant across treatments at P_{CO_2} = 0.3 Torr. Oxygen concentrations were monitored in aquaria with a polarographic silver–platinum O_2 electrode (YSI model 53). Oxygen measurements were determined several times a week for the duration of each hypoxia exposure treatment period.

Exposure of samples to hypoxia was maintained for at least 720 h (30 days) or until 50% mortality in all replicate treatments was achieved. Mortality was checked and water replaced in all aquaria at 24-h intervals throughout the exposure periods. Replacement water was maintained in the environmental chamber with treatment aquaria. Replacement hypoxic water was created by bubbling a water-filled carboy with mixed gas supplies identical to those used to maintain hypoxia in treatment aquaria for 24 h before use. Zebra mussels were considered dead when they failed to respond to stimulation of their posterior mantle edge, siphons, or foot with a blunt probe. Byssal attachment was monitored daily to determine if the mussels abandoned their byssus prior to death. Viability in Asian clams was monitored by gently forcing the tip of a blunt dissection needle between the edge of the valves at the posterior or ventral shell margin. Asian clams do not always gape upon death, and if the valves failed to close after probe entry, the animal was considered dead. Viability monitoring was conducted while specimens were under hypoxic water to minimize exposure to air and elevated O_2 levels.

The prolonged hypoxic exposures necessary to produce measurable mortality in both *D. polymorpha* and *C. flumenia* presented some complications with the data analysis. The lack of 100% mortality in several of the high-P_{O_2} treatments (i.e., 15.9 and 23.8 Torr) or those held at lower temperatures (15 and 5°C) required a statistical analysis that could incorporate censored variables (Parmar and Machin 1995). In these analyses, the death of an individual is considered an event, and any survivors at the end of the exposure period are censored data. All censored data points are incorporated into the final analyses and not discarded, so sample sizes are equal among treatments (n = 90). We applied several nonparametric analyses, that incorporate censored variables to contrast survivorship among hypoxia and temperature treatments. The LT_{50} values for each species and each exposure treatment with at least 50% mortality were calculated with the Kaplin–Meier survivorship analysis (SPSS Inc. 1993). The Kaplan–Meier survivorship statistic evaluates the survival curve only when an event occurs, so probabilities are not calculated at censored times (Parmar and Machin 1995). Because hypoxic exposures were quite long in some treatments, there was usually some mortality (1–6%) in the normoxic controls. Control sample mortality precluded the use of the Spearman–Karber method for LT_{50} estimates, which assumes no control mortality during the exposure period (Newman 1994). Survivorship differences between O_2 and temperature treatments were determined with the log-rank statistic (Mantel–Cox) test (SPSS Inc. 1993). All statistical tests used a probability level of ≤0.05 to determine significance among hypoxia and temperature treatments.

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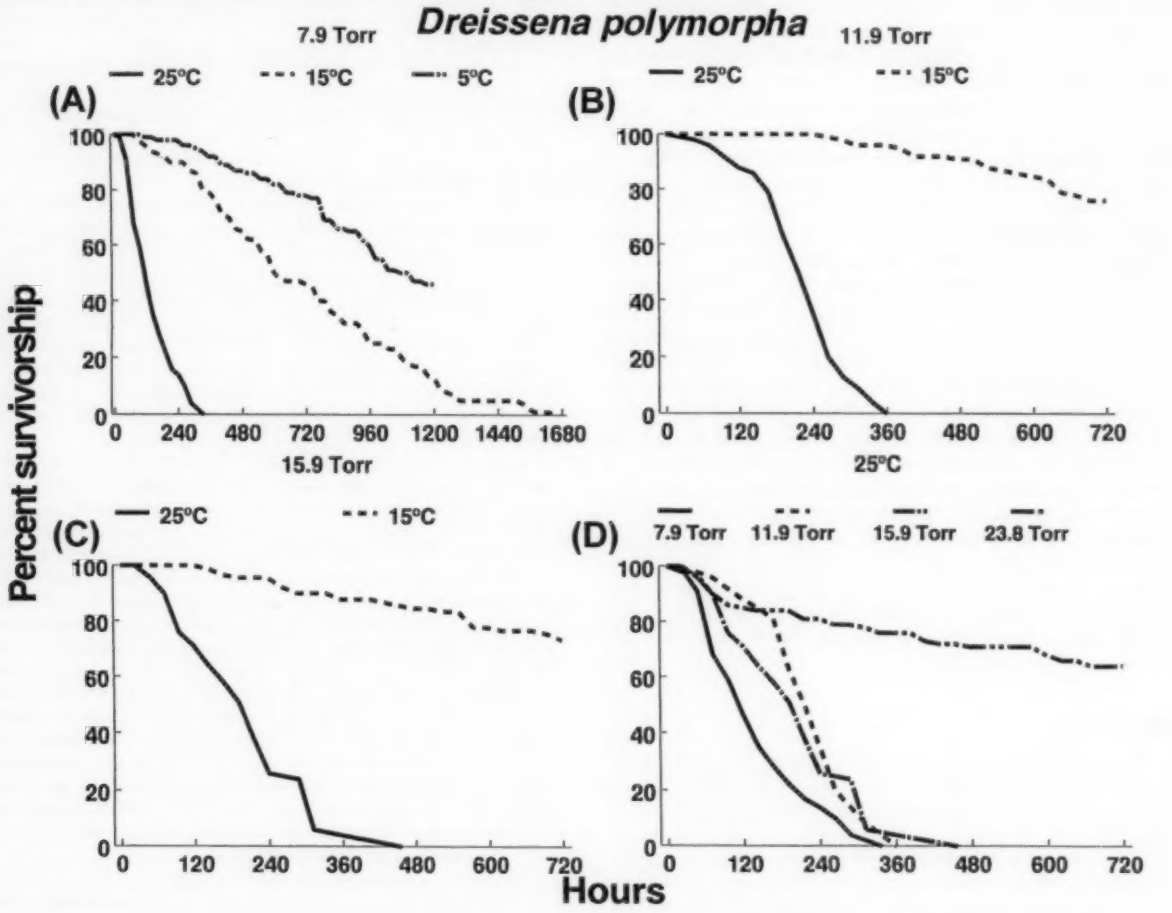
Fig. 1. Actual percent survival over time for *D. polymorpha* to constant hypoxic stress of (A) 7.9-Torr Po_2 at 5, 15, and 25°C, (B) 11.9-Torr Po_2 at 15 and 25°C, (C) 15.9-Torr Po_2 at 15 and 25°C, and (D) 7.9-, 11.9-, 15.9-, and 23.8-Torr Po_2 at 25°C. Survivorship curves represent the mean survivorship of all replicates combined ($n = 3$) for each temperature and Po_2 treatment.



The time-dependent Cox proportional hazards model was also used to evaluate survivorship differences among temperature and hypoxia treatments. The Cox proportional hazards model is a regression analysis that evaluates the effects of several independent variables (covariates) on the dependent variable (survivorship). We used the Cox proportional hazards model to evaluate the effect of bivalve size on survivorship, in addition to O_2 concentration and test temperature. A multiple linear regression cannot be used for analysis of time to event data with censored data because censored observations cannot be incorporated into the model, nor can they be ignored (Cox and Oakes 1984). The Cox proportional hazards model calculates a regression coefficient (B) and a Wald statistic for each covariate and their interactions. The Wald statistic is the ratio of the covariate coefficient to its standard error, squared (SPSS Inc. 1993). These analyses are unusual for ecological applications, but are used extensively by medical researchers (Parmar and Machin 1995) and were appropriate for the requirements of this investigation.

Results

Dreissenia polymorpha survivorship

Survivorship for both species was reduced by exposure to hypoxic treatments, compared with mortality rates among control treatments ($Po_2 = 159$ Torr). The Kaplan–Meier LT_{50} estimates for *D. polymorpha* indicated that survivorship was greater with increasing O_2 concentration and decreasing temperature (Fig. 1; Table 1). Log-rank analysis of *D. polymorpha* survivorship at 7.9 Torr indicated significant differences ($p < 0.05$) in survivorship among temperature treatments (Table 2). The 7.9-Torr survivorship for *D. polymorpha* in the 15 and 5°C treatments was five to nine times greater than recorded for the 25°C treatment (Table 2). In comparison, control samples held at 159 Torr (100% air O_2 saturation) showed 0, 3, and 6% mortality over the same exposure periods for the respective 5, 15, and 25°C treatments. Cox regression analysis indicated that there were significant differences in survivorship between temperatures and mussel size (shell length) (Table 3).

Dreissena polymorpha survivorship at 11.9 Torr-Po_2 for 15 and 25°C was greater than the survivorship of samples held at 7.9 Torr at the same temperatures (Table 1; Fig. 1). The 11.9-Torr treatment exhibited only 26% mortality during the 720-h exposure period at 15°C (Table 1; Fig. 1B). Log-rank analysis

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Table 1. Kaplan–Meier LT_{50} survival estimates for each treatment and replicate of *D. polymorpha* and *C. fluminea* under different combinations of temperature and hypoxia.

O_2 tension (Torr)	Temperature (°C)	Replicate	Shell length (mm)	LT_{50}	95% CI
			Dreissena polymorpha		
7.9	5	1	21.6 (17.4–26.7)	1152.00	865.13–1356.08
		2	21.0 (15.5–27.2)	912.00	589.94–1234.06
		3	20.8 (13.6–26.4)	960.00	659.41–1260.59
	15	1	21.5 (15.5–26.5)	408.00	343.59–472.41
		2	21.1 (15.7–26.7)	792.00	467.26–1116.74
		3	21.4 (15.3–25.7)	768.00	574.76–961.24
	25	1	18.9 (13.4–26.4)	120.00	55.73–184.27
		2	21.0 (16.2–28.4)	120.00	102.26–137.74
		3	20.3 (13.6–26.7)	144.00	123.31–164.69
11.9	15	1	20.8 (16.5–26.7)	17% mortality at 720 h	
		2	21.5 (16.2–29.1)	26% mortality at 720 h	
		3	22.3 (15.6–31.2)	44% mortality at 720 h	
	25	1	23.5 (16.7–33.2)	264.00	246.65–281.35
		2	21.1 (15.7–27.6)	216.00	188.46–243.54
		3	23.3 (17.5–32.6)	216.00	200.48–231.52
15.9	15	1	22.1 (14.6–28.7)	13% mortality at 720 h	
		2	21.7 (14.3–28.4)	60% mortality at 720 h	
		3	22.3 (14.3–32.2)	10% mortality at 720 h	
	25	1	19.5 (15.6–26.2)	240.00	205.30–274.70
		2	19.1 (15.2–24.5)	168.00	119.69–216.31
		3	20.1 (15.0–25.2)	192.00	159.87–224.13
23.8	25	1	21.6 (14.8–30.1)	33% mortality at 720 h	
		2	23.3 (15.3–29.9)	33% mortality at 720 h	
		3	23.2 (15.4–29.4)	33% mortality at 720 h	
			Corbicula fluminea		
7.9	5	1	19.4 (16.2–22.4)	560.00	511.98–608.02
		2	19.7 (16.6–25.7)	432.00	380.47–483.53
		3	18.6 (16.3–21.9)	480.00	443.52–516.48
	15	1	18.8 (13.6–22.0)	480.00	448.93–511.07
		2	19.3 (16.4–22.0)	480.00	445.30–514.70
		3	18.6 (15.2–24.1)	480.00	470.92–489.08
	25	1	19.1 (16.5–24.0)	144.00	118.47–169.53
		2	20.0 (17.2–23.8)	96.00	80.04–111.96
		3	19.9 (16.3–26.0)	168.00	160.41–175.59
11.9	15	1	19.2 (17.0–21.5)	408.00	376.08–439.92
		2	20.2 (16.5–24.0)	408.00	344.16–471.84
		3	20.5 (17.3–23.5)	360.00	317.44–402.56
	25	1	19.8 (14.1–23.4)	192.00	127.59–256.41
		2	20.5 (17.8–23.8)	216.00	198.65–233.35
		3	20.1 (17.9–25.2)	216.00	179.52–252.48
15.9	15	1	18.8 (16.7–22.1)	264.00	238.24–289.76
		2	19.4 (16.3–22.7)	216.00	205.36–226.64
		3	20.1 (17.9–25.2)	360.00	317.44–402.56
	25	1	18.8 (16.7–22.1)	120.00	68.47–171.53
		2	19.4 (16.3–22.7)	264.00	135.18–392.82
		3	20.1 (17.9–25.2)	216.00	179.52–252.48

Note: Replicates of each species contained 30 individuals and mean shell length and size range are given. The median LT_{50} value is presented along with the 95% confidence interval for each replicate sample.

determined survivorship at 15°C to be significantly greater than that at 25°C (Table 2). There was no mortality associated with the normoxic ($Po_2 \approx 159$ Torr) control samples, exposed concurrently with the 11.9-Torr treatments. Cox regression analysis indicated significant differences in survivorship between the 15 and 25°C treatments (Table 3). Additionally, zebra mussel size significantly affected time to death in the 11.9-Torr treatments, with larger animals dying more rapidly (Table 3).

Dreissena polymorpha survivorship at 15.9 Torr at 15 and

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Table 2. Results of the log-rank statistic test on the survivorship of *D. polymorpha* and *C. fluminea* to different levels of hypoxic exposure at three different temperatures.

O_2 tension (Torr)	Temperature (°C)	LT_{50}	log-rank	SM_{100}
		Dreissena polymorpha		
7.9	25	120.00	A	336.00
	15	600.00	B	1656.00
	5	1056.00	C	54% mortality at 1200 h
11.9	25	261.00	A	384.00
	15		B	26% mortality at 1200 h
15.9	25	216.00	A	456.00
	15		B	28% mortality at 720 h
7.9	25	120.00	A	336.00
11.9	25	216.00	B	384.00
15.9	25	216.00	B	456.00
23.8	25		C	34% mortality at 720 h
31.8	25			2% mortality at 720 h
		Corbicula fluminea		
7.9	25	144.00	A	408.00
	15	480.00	B	576.00
	5	480.00	C	816.00
11.9	25	216.00	A	360.00
	15	384.00	B	600.00
15.9	25	216.00	A	408.00
	15	288.00	B	528.00
7.9	25	144.00	A	408.00
11.9	25	216.00	B	360.00
15.9	25	216.00	B	408.00
23.8	25			3% mortality at 720 h

Note: The median LT_{50} value for all individuals ($n = 90$) across all replicate samples ($n = 3$) is given for reference. The SM_{100} value is also given to directly compare maximal survivorship at different hypoxia and temperature treatments.

25°C was improved over lower O_2 concentration (Fig. 1). Survivorship estimated as LT_{50} values ranged from 231.5 to 281.4 h among replicate samples at 25°C and survivorship improved at 15°C with only 28% mortality after a 720-h exposure period (Table 1). The log-rank statistic determined survivorship differences between the 15 and 25°C exposures (Table 2). Control samples held at 159 Torr concurrently with the 15.9-Torr exposures exhibited 3 and 1% mortality for the 25 and 15°C samples experiencing identical exposure periods. Cox regression analysis indicated a significant size effect, with larger animals dying more rapidly than smaller animals across treatments (Table 3).

Survivorship of *D. polymorpha* for the 23.8-Torr Po_2 exposure at 25°C was greatly increased over treatments (Table 1). Only 34% mortality occurred across replicates during a 720-h continuous exposure period (Fig. 1). Log-rank analysis determined that mortality was significantly lower in the 23.8-Torr treatment compared with all other 25°C treatments (Table 2). Control exposures (159 Torr) concurrent with the 23.8-Torr treatment exhibited only 5% mortality during the same 720-h period.

Dreissena polymorpha exhibited 2% mortality during a 720-h exposure to a Po_2 of 31.8 Torr (20% air O_2 saturation) at 25°C (Table 1). The 31.8-Torr treatment mortality was identical to the mortality rate of control samples held at 159 Torr during the same period. The lack of mortality associated with the 31.8-Torr exposure level precluded further data analysis for this treatment.

Cox regression analysis contrasted 25°C survivorship of *D. polymorpha* at four different hypoxic exposures, and the 7.9-, 11.9-, and 15.9-Torr treatments were individually contrasted against the 23.8-Torr level in this analysis (Table 3). Cox regression analysis indicated that *D. polymorpha* survivorship at 23.8 Torr was significantly greater than recorded under all other 25°C exposure treatments (Table 3). Additionally, Cox regression analysis revealed that zebra mussel size had a significant effect on survivorship for the 7.9-Torr treatment (Table 3). The general trend was that mortality occurred earlier in larger specimens. Size by hypoxia level interactions indicated that the effect of size on survivorship was reduced as O_2 concentration increased (Table 3).

Dreissena polymorpha did not abandon their byssus in response to hypoxic stress at any tested level of hypoxia or temperature. Few mussels (usually fewer than three) were detached during any monitoring period for any hypoxic and temperature treatment. Detached mussels were usually individuals that were previously attached, but had to be removed from another individual that had died during an earlier monitoring period.

Corbicula fluminea survivorship

The survivorship of *Corbicula fluminea*, like that of *D. polymorpha*, was reduced with decreasing O_2 concentration and increasing temperature. Specifically, 7.9-Torr exposures had increasing survivorship in the lower temperature treatments (Fig. 2). At 5°C, survivorship was more variable among replicate samples than exposures were for either the 15 or 25°C treatment (Table 1). The Kaplan–Meier LT_{50} values determined for the overall sample medians at 5 and 15°C were an identical 480 h (Table 2). The 7.9-Torr Po_2 treatment at 25°C had a median LT_{50} of 144 h (Table 2), and replicate samples were again variable (Table 1). However, log-rank analysis determined that survivorship was different between the different temperature treatments (Table 2; Fig. 2). *Corbicula fluminea* controls experienced a higher mortality rate (16%) in the 5°C control ($Po_2 \approx 159$ Torr) than they did in the 15 and 25°C controls combined (3 and 6%, respectively). Additionally, Cox regression analysis of the 7.9-Torr data indicated that survivorship in the 15 and 5°C treatments was significantly greater than in the 25°C treatment (Table 3). Mussel size (shell length) significantly affected survivorship, with larger animals displaying reduced survivorship, and no size treatment interaction was detected (Table 3).

Corbicula fluminea survivorship under the 11.9-Torr hypoxia treatment was different among the 15 and 25°C treatments (Fig. 2). Survivorship at 15°C was variable among replicates (Table 1), and the median LT_{50} was 384 h (Table 2). The 25°C exposure had significantly reduced survivorship, with a median LT_{50} of 216 h (Table 2). In addition, the 11.9-Torr treatment displayed minimal variation in survivorship between replicates (Table 1), and the log-rank statistic determined survivorship between the 15 and 25°C treatments at 11.9 Torr to be significantly different (Table 2). The 159-Torr control exposure samples had no mortality associated with either the 15 or 25°C treatment. Cox regression analysis

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Table 3. Results of the Cox regression analysis on the survivorship of *D. polymorpha* and *C. fluminea* samples at 7.9, 11.9, and 15.9 Torr and at different hypoxic treatments at 25°C.

Variable	df	Wald statistic	*B*	*p*	*R*
		Dreissena polymorpha			
7.9 Torr					
Temperature	2	181.41		0.00001	0.28
5°C	1	179.60	–3.81	0.00001	0.28
15°C	1	126.37	–2.92	0.00001	0.23
Shell length	1	0.01	0.01	0.93	0.00
11.9 Torr					
Temperature	1	66.62	–4.31	0.00001	–0.24
Shell length	1	8.05	–0.08	0.00001	0.07
Shell length × temperature	1	0.01	0.01	0.93	0.00
15.9 Torr					
Temperature	1	104.45	–3.58	0.00001	–0.30
Shell length	1	11.05	0.11	0.0008	0.09
Shell length × temperature	1	0.90	–0.06	0.34	0.00
25°C					
O_2	3	26.09		0.00001	0.08
7.9 Torr	1	22.23	7.06	0.00001	0.08
11.9 Torr	1	8.86	4.70	0.00001	0.046
15.9 Torr	1	6.05	3.83	0.003	0.035
Shell length	3	8.93	0.16	0.003	0.046
Shell length × O_2	3	11.93		0.008	0.042
Shell length × 7.9 Torr	1	8.06	–0.18	0.045	–0.043
Shell length × 11.9 Torr	1	2.61	–0.10	0.105	–0.014
Shell length × 15.9 Torr	1	0.35	–0.04	0.549	0.000
		Corbicula fluminea			
7.9 Torr					
Temperature	2	218.00		0.00001	0.28
5°C	1	208.67	–3.42	0.00001	0.28
15°C	1	157.01	–2.70	0.00001	0.23
Shell length × temperature	1	0.09		0.95	0.00
Shell length	1	6.66	–0.08	0.01	–0.04
Shell length × 5°C	1	0.02	–0.01	0.89	0.00
Shell length × 15°C	1	0.09	–0.02	0.76	0.00
11.9 Torr					
Temperature	1	106.68	–2.33	0.00001	–0.24
Shell length	1	0.94	0.44	0.33	0.00
15.9 Torr					
Temperature	1	26.79	–0.89	0.00001	–0.12
Shell length	1	4.15	0.09	0.04	0.09
Shell length × temperature	1	5.19	–0.19	0.02	–0.04
25°C					
O_2	2	16.89		0.0002	0.07
7.9 Torr	1	15.76	0.62	0.0001	0.08
11.9 Torr	1	1.40	0.18	0.23	0.00
Shell length	1	1.09	0.03	0.29	0.00
Shell length × O_2	2	0.09	0.95	0.95	0.00
Shell length × 7.9 Torr	1	0.01	0.89	–0.01	0.00
Shell length × 11.9 Torr	1	0.09	0.76	–0.02	0.00

also determined that survivorship was significantly influenced by temperature but that clam size was not a factor in the 11.9-Torr treatments (Table 3).

Survivorship in the 15.9-Torr treatment for *C. fluminea* at 15 and 25°C was also significantly different among temperatures (Table 2; Fig. 2). The Kaplan–Meier LT_{50} median estimates for the 15.9-Torr treatment were similar to those for the 11.9-Torr exposure, with 216 and 288 h for the 25 and 15°C treatments, respectively. The log-rank statistic determined significant differences in survivorship between the 15 and 25°C treatments (Table 2). Asian clam mortality in the concurrent normoxic controls (P_{O_2} = 159 Torr) was 1% for

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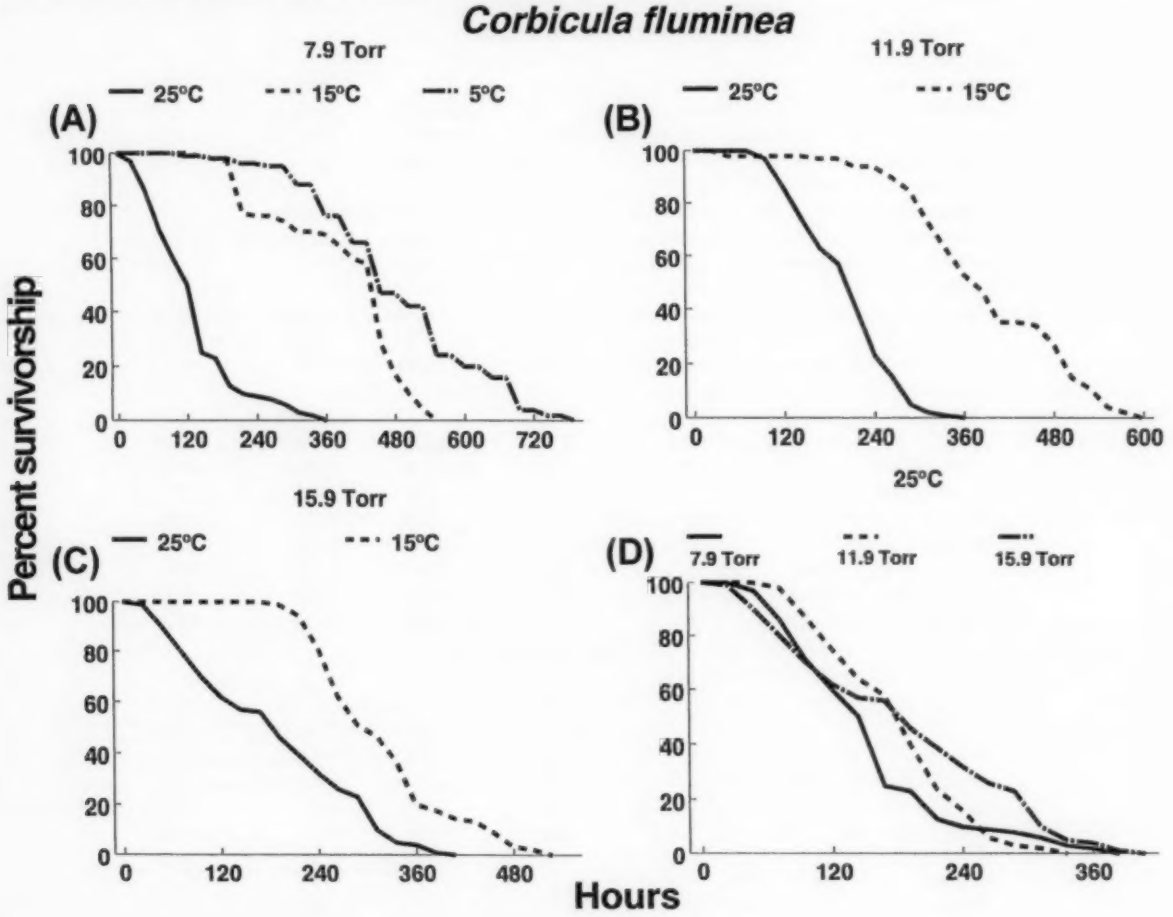
Fig. 2. Actual percent survival over time for *C. fluminea* to constant hypoxic stress of (A) 7.9-Torr Po_2 at 5, 15, and 25°C, (B) 11.9-Torr Po_2 at 15 and 25°C, (C) 15.9-Torr Po_2 at 15 and 25°C, and (D) 7.9-, 11.9-, and 15.9-Torr Po_2 at 25°C. Survivorship curves represent the mean survivorship of all replicates combined ($n = 3$) for each temperature and Po_2 treatment.



both the 15 and 25°C treatments. Cox regression analysis for the 15.9-Torr data also determined survivorship differences with respect to both temperature, shell length, and their interaction (Table 3).

Survivorship differences across hypoxia treatments and replicates at 25°C for *C. flumenia* are given in Table 1. Log-rank analysis determined significant survivorship differences for the 7.9-Torr treatment but not for the 11.9- and 15.9-Torr treatments (Table 2). Survivorship improved dramatically in the 23.8-Torr exposure level, with only 3% mortality after a 720-h exposure period (Table 2). The low mortality rate for the 23.8-Torr, 25°C treatment precluded further data analysis. The 23.8-Torr control samples held at 159 Torr displayed 2% mortality over the same 720-h exposure period. Cox regression analysis determined that survivorship was significantly different between the 7.9- and 15.9-Torr treatments but not between the 11.9- and 15.9-Torr treatments at 25°C (Table 3). The Cox regression also detected a size effect for the 25°C hypoxic exposures, but no interactions were significant (Table 3), with larger animals expiring more quickly.

Discussion

The capacity of these bivalves to withstand prolonged hypoxic exposure could be explained by a number of factors. First, O_2 regulatory ability or metabolic rate changes with temperature and will influence survivorship. Second, results for *C. fluminea* were affected by cold temperature as well as hypoxia (high mortality in 5°C controls). Third, sensitivity to hypoxia does display seasonal trends, which reflects variability in the general physiological condition of both species. Finally, the hypoxia survivorship data, presented in this study, help clarify the habitat distributions and requirements of both species.

Low-anoxia survivorship in *D. polymorpha* was first reported by Mikheev (1964), who reported 3-, 4-, and 6-day survivorship for zebra mussels held in complete anoxia at 23, 20, and 17°C, respectively. However, Matthews and McMahon (1995) found that the anoxia survivorship of *D. polymorpha* was 60 h at 25°C, 330 h at 15°C, and 905 h at 5°C. The differences in zebra mussel survivorship between the two

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studies were likely the result of the closed systems utilized by Mikheev (1964) in which treatment water was not replaced for the duration of the experiment. The increased survivorship of *D. polymorpha* that we recorded with decreasing temperature and increasing O_2 concentration indicates this species has a limited capacity for O_2 regulation. The O_2 consumption rate of *D. polymorpha* decreases with decreasing temperature, and its O_2 regulatory capacity increases on acclimation to lower temperatures (5 and 15°C) (McMahon 1996). Thus, temperature-dependent metabolic responses of *D. polymorpha* correlate with the increasing hypoxia tolerances recorded at lower test temperatures.

In contrast, *C. fluminea* survivorship under anoxia at 5, 15, and 25°C (Matthews and McMahon 1995) fell within the survivorship range recorded under various levels of hypoxia for this species at corresponding test temperatures. *Corbicula fluminea* was not tolerant of extreme hypoxia ($Po_2 \leq 15.9$ Torr), but increased survivorship above 15.9 Torr, indicating that this species has a critical Po_2 level. Additionally, lower temperatures improved *C. fluminea* survivorship only marginally in contrast with *D. polymorpha*. Overall, *C. fluminea* proved more tolerant of anoxia (Matthews and McMahon 1995) but less tolerant of hypoxia than *D. polymorpha*. Kapper and Stickle (1987), Stickle et al. (1989), and Das and Stickle (1993) have documented similar anoxia and hypoxia responses in the marine molluscs *S. haemastoma* and *C. virginica*. The differing responses of *D. polymorpha* and *C. fluminea* to hypoxia and anoxia, like those of marine molluscs (Kapper and Stickle 1987; Stickle et al. 1989), suggest that the capacity of a particular mollusc species to tolerate anoxia may not directly reflect its hypoxia tolerance. The limited effect of decreasing temperature on *C. fluminea* survivorship, in addition to a discrete hypoxia limit ($Po_2 \leq 15.9$ Torr), indicates that this species has a reduced regulatory capacity when contrasted with *D. polymorpha*.

The nonprotein organic energy stores of both *D. polymorpha* (Sprung 1995) and *C. fluminea* (Williams and McMahon 1989) vary seasonally, with minimal concentrations in summer and maximal concentrations during the winter. As carbohydrate energy stores are utilized to support anaerobic metabolism, summer-conditioned bivalves may not be able to tolerate lethal levels of hypoxia for as long as winter-conditioned bivalves. Thus, the poor nutritional condition of summer bivalves may have contributed to the reduced survivorship times in 25°C hypoxic treatments and could explain the variability in survivorship among similar hypoxic concentrations at different temperatures. In the future, investigators studying resistance to physiological stress or toxins with these species should be aware that seasonal variation in condition may affect experimental outcomes.

The temperature-dependent hypoxia survivorship for *D. polymorpha* and the strict limitation of *C. fluminea* to Po_2 levels above 23.8 Torr support the distributional information reported for both species (Counts 1986; Mills et al. 1993; Stańczykowska and Lewandowski 1993). *Corbicula fluminea* is restricted to shallow, well-oxygenated habitats that remain between 2 and 36°C (McMahon 1983; Counts 1986). Additionally, low temperatures have been responsible for massive winter kills in northern fringe populations of *C. fluminea* (Cherry et al. 1980) and explain the high mortality recorded in the 5°C controls in this study. Indeed, *C. fluminea* is most dense in the southern and southwestern United States (Counts 1986), implying that this species prefers warm waters that do not undergo long periods of anoxic or hypoxic stress. In contrast, *D. polymorpha* is unable to withstand prolonged exposure to waters above 30°C (McMahon 1996) and is most dense at depths less than 20 m (Stańczykowska and Lewandowski 1993). Dermott and Munawar (1993) reported *D. polymorpha* abundant at 50 m in Lake Erie, where temperatures did not exceed 5°C. However *D. polymorpha* was specifically excluded from the profundal zone in western Lake Erie, which experiences regular anoxia in the summer months (Dermott and Munawar 1993). The capacity of zebra mussels to increase O_2 regulatory ability at lower temperatures allows overwinter survival in aquatic habitats made hypoxic by ice cover. The ability of *D. polymorpha* to survive and grow in profundal sediments indicates a capacity for the regulation of O_2 consumption at low hypolimentic temperatures, as long as the habitat does not undergo long-term anoxia/hypoxia, during summer months.

Finally, published Q_{10} values for both species (McMahon 1979, 1996) support survivorship data from this study by demonstrating the greatly reduced metabolic demands of both bivalve species at lower temperatures. The Q_{10} values for *C. fluminea* computed from McMahon (1979) were 2.5, 2.5, and 3.5, respectively, for 10, 20, and 30°C acclimated animals. *Dreissena polymorpha* Q_{10} values were 2.3, 2.6, and 3.8, respectively, for 5, 15, and 25°C acclimated individuals (McMahon 1996). As both species display limited capacities for temperature-compensated O_2 uptake rate (McMahon 1979, McMahon 1996), the relatively high Q_{10} values suggest that metabolic demands are greatly reduced with decreasing temperature, explaining the prolonged survivorship at lower temperatures recorded in this study. The relatively poor hypoxia tolerance of both species correlates well with their general exclusion from chronically hypoxic or anoxic waters. Our results suggest that O_2 deprivation measures for the macrofouling control for both species will require very long application times that will be exacerbated by decreasing temperature. Further, slight increases in O_2 concentration above complete anoxia prolong tolerance times in *D. polymorpha*, but would have a reduced impact on *C. fluminea* survivorship. However, this treatment approach could be very effective during late-summer periods when animals are more likely to be seasonally stressed (Williams and McMahon 1989; Sprung 1995).

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Crustacean zooplankton species richness: single- and multiple-year estimates

Shelley E. Arnott, John J. Magnuson, and Norman D. Yan

Abstract: Richness estimates are dependent on the spatial and temporal extent of the sampling programme and the method used to predict richness. We assessed crustacean zooplankton richness in eight Canadian Shield lakes at different temporal and spatial scales using three methods of estimation: cumulative, asymptotic, and Chao's index. Percent species detected increased with the number of spatial, intraannual, or interannual samples taken. Single samples detected 50% of the annual species pool and 33% of the total estimated species pool. This suggests that previous estimates of zooplankton richness, based on single samples in individual lakes, are too low. Our richness estimates for individual lakes approach the total number of zooplankton found in some regions of Canada, suggesting that each lake has most taxa at some time, the majority being very rare. Single-year richness estimates provided poor predictions of multiple-year richness. The relationship between richness and environmental variables was dependent on the method of estimation and the number of samples used. We conclude that richness should be treated as an "index" rather than an absolute and sampling efforts should be standardized. We recommend an asymptotic approach to estimate zooplankton richness because the number of samples taken influenced it less.

Résumé : Les estimations de la diversité dépendent de l'étendue spatiale et temporelle du programme d'échantillonnage et de la méthode utilisée pour prévoir la diversité. Nous avons évalué la diversité des crustacés du zooplancton dans huit lacs du Bouclier canadien à des échelles temporelles et spatiales différentes en utilisant trois méthodes d'estimation, soit les méthodes cumulative, asymptotique et de l'indice de Chao. Le pourcentage d'espèces détecté s'accroissait avec le nombre d'échantillons spatiaux, intra-annuels et inter-annuels prélevés. Les échantillons simples ont détecté 50% du bassin annuel d'espèces et 33% du bassin d'espèces total estimé. Cela laisse penser que les estimations antérieures de la diversité du zooplancton, fondées sur des échantillons simples prélevés dans un lac, sont trop faibles. Nos estimations de la diversité pour les lacs pris individuellement s'approchent du nombre total d'espèces zooplanctoniques trouvées dans certaines régions du Canada, ce qui laisse penser que chaque lac renferme la plupart des taxons à un certain moment, la majorité étant très rares. Les estimations de la diversité sur une seule année fournissent de mauvaises prévisions de la diversité sur plusieurs années. La relation entre la diversité et les variables environnementales dépendait de la méthode d'estimation et du nombre d'échantillons utilisés. Nous concluons que la diversité obtenue devrait être vue comme un « indice » plutôt que comme une valeur absolue, et que les efforts d'échantillonnage devraient être normalisés. Nous recommandons l'emploi d'une méthode asymptotique pour évaluer la diversité du zooplancton parce que le nombre d'échantillons prélevés a moins d'effet avec cette méthode.

[Traduit par la Rédaction]

Introduction

Increased biological impoverishment of freshwater ecosystems, resulting from human activities such as habitat destruction, release of toxic materials, and introduction of exotic species, has heightened the importance of assessing biodiversity and understanding its connections with ecosystem function (Carpenter et al. 1992; Schulze and Mooney 1993). Obtaining a reliable and repeatable estimate of biodiversity is a critical element in all such endeavors. However, many issues associated with developing acceptable methods of estimating the number of species in a particular habitat or ecosystem are unresolved (Harper and Hawksworth 1994).

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One such issue is that biodiversity is a property that cannot be measured with absolute certainty because we can rarely census an entire community. Therefore, we must determine the sampling effort and temporal and spatial scale at which biodiversity should be assessed. Sampling issues may be particularly important in aquatic habitats because of their small-scale patchiness and short-term temporal variability. Methodological factors certainly influence estimates of species richness in aquatic habitats (Minns 1989; Magnuson et al. 1994).

Estimated species richness increases with the number of individuals or samples collected (Fisher et al. 1943). Several techniques that exploit this relationship have been developed, including rarefaction (standardizing to a common number of individuals) (Hurlbert 1971), species accumulation curves (Miller and Wiegert 1989), species–area curves, integration of lognormal distributions, and jackknife and bootstrap resampling methods (Heltshe and Forrester 1983). These methods are primarily directed toward addressing the influence of increasing the number of individuals collected, but few studies have explicitly investigated how richness estimates are influenced by the spatial and temporal extent of the sampling routine.

Zooplankton populations exhibit substantial spatial (e.g., Pinel-Alloul and Pont 1991) and temporal heterogeneity

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Table 1. Morphometric and chemical data (SD in parentheses) for the study lakes during the period 1978–1989.

Lake	Latitude (N)	Longitude (W)	Area (ha)	Depth (m)		Alkalinity (mg $CaCO_3$/L)	Conductivity (μS)	DOC (mg/L)	pH	TP (μg/L)	No. of fish species	*Chaoborus* (no.·m^{-3})
				Mean	Maximum							
Blue Chalk	45°11′	78°56′	52.4	8.5	23.0	4.09 (0.34)	28.92 (0.67)	1.79 (0.20)	6.65 (0.15)	6.30 (0.87)	9	0.7
Chub	45°13′	78°59′	34.4	8.9	27.0	0.81 (0.23)	27.87 (0.65)	4.67 (0.28)	5.62 (0.14)	10.39 (1.42)	4	35.9
Crosson	45°05′	78°02′	56.7	9.2	25.0	0.55 (0.18)	25.99 (0.46)	4.03 (0.23)	5.58 (0.11)	11.00 (1.42)	8	0.4
Dickie	45°09′	79°05′	93.6	5.0	12.0	1.15 (0.41)	28.29 (0.77)	4.97 (0.33)	5.86 (0.17)	11.38 (2.07)	9	10.7
Harp	45°23′	78°08′	71.4	13.3	37.5	3.29 (0.20)	34.40 (0.65)	3.81 (0.24)	6.29 (0.10)	7.52 (1.08)	9	0.4
Heney	45°08′	79°06′	21.4	3.3	5.8	0.51 (0.22)	25.03 (0.81)	2.82 (0.26)	5.81 (0.17)	7.04 (1.38)	7	0
Plastic	45°11′	78°50′	32.1	7.9	16.3	0.52 (0.19)	22.48 (0.57)	2.22 (0.14)	5.74 (0.13)	5.96 (0.98)	9	4.4
Red Chalk	45°11′	78°56′	32.1	16.7	38.0	3.31 (0.24)	29.39 (0.40)	2.45 (0.19)	6.33 (0.16)	5.06 (0.71)	9	0

(Sommer et al. 1986). The primary goal of this paper is to evaluate how this patchiness influences assessments of species richness in lakes. Because of this patchy nature of zooplankton populations, we would expect that richness estimates would be highly influenced by both the spatial and temporal extent of the sampling programme. Single samples taken at one location in the lake or on one date may not adequately represent the total species richness of the community.

We present estimates of crustacean zooplankton species richness at several different temporal and spatial scales: multiple stations within a lake, multiple samples within a year, and multiple-year samples. From the results of these comparisons, we suggest a method of estimating zooplankton species richness and develop a strong rationale for the idea that standardized methods must be used to arrive at indices. That is, sample richness and total richness do not converge, even with a relatively large number of samples, and the difference between them is highly dependent on the number of samples taken. The level of sampling effort must be standardized to obtain meaningful comparisons among sites.

Methods

Study lakes

The eight study lakes are located on the Canadian Shield in south-central Ontario, Canada, near the Dorset Environmental Science Centre. The watersheds are forested (mixed deciduous and coniferous) and nonagricultural, owing to rough topography, thin soils, and cool climate. Lakes differ in characteristics important to the ecology of Shield lakes, including size, trophy, alkalinity, presence of glacial relicts, and invertebrate predator and fish populations (Table 1).

Zooplankton sampling

Although the sampling protocol was not designed for biodiversity research (data were collected as part of a biomonitoring programme), we believe that analysis of this database can provide useful insights into sampling issues associated with the estimation of species richness. The primary strength of the Dorset zooplankton database is that methods have remained consistent since 1978, a longer period than for most studies. Lakes were sampled at the same location using the same gear deployed in the same manner under the direction of the same crew chief. Samples were preserved, composited, and subsampled in the same way and counted using the same protocol by the same person (Yan et al. 1996).

Each lake was sampled from six to 27 times each year during the ice-free season from 1978 to 1989. For multiple-year analyses, we standardized the database to six samples per year for each lake. When more than six samples were taken in a year, we randomly chose one sample per month during the ice-free season. When there were more than six months when samples were collected, we used data from months closest to the summer season (May–October).

Zooplankton were collected with a metered plankton net (McQueen and Yan 1993) at a single station located at the deepest area in the lake. The conical net had a mesh size of 80 μm and a mouth diameter of 12.5 cm. It was equipped with a unidirectional current meter. Sample volumes were calculated using measured haul filtration efficiencies, which averaged 81%.

The net was hauled from several different depths to the surface (Yan et al. 1996) and the contents of the hauls were combined. There are large, often persistent vertical differences in zooplankton abundance, and community composition is often related to thermal stratification. Samples were composited such that vertical strata contributed to the sample in proportion to strata volumes. This was done so that samples would not be biased toward organisms occupying

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lower depths, nor would the epilimnion be underrepresented. Samples were preserved with 6% buffered sugar–formalin. A minimum of 250 crustacean zooplankton was counted in each sample. A Folsom plankton splitter was used to generate subsamples of various sizes. The larger subsamples were used to count rare, large animals whereas the smaller subsamples were used to count the most abundant species. In this way, no single species contributed more than 20% of the total count. All Cladocera and mature Copepoda were identified to species. We combined counts of *Diaphanosoma brachyurum* with *Diaphanosoma birgei* to allow for changes in nomenclature over the course of the study (Korínek 1981). *Daphnia pulicaria* and *Daphnia pulex* were combined and enumerated as *D. pulex* because we did not believe that they could be reliably distinguished (Dodson 1981). *Eubosmina longispina* was excluded from our analysis because its identification was not consistent throughout the entire database. Naupliі and other unidentifiable juveniles (mostly copepods) were excluded from our analyses.

Species richness was estimated at several temporal scales using three different methods of calculation. For clarity, we use a standard notation throughout the paper: $S_{\text{no. of years, no. of samples within a year, index}}$. For example, $S_{12,6,\text{cum}}$ is based on 12 years and six samples within each year using the cumulative richness estimate.

Single-year richness

Multiple stations within a lake

Ten stations, including the central station, were sampled once in May, June, July, and August 1985 in Plastic Lake. The stations were uniformly distributed throughout the lake at depths ranging from 5 to 16 m. A single integrated sample was taken by towing a plankton net from the bottom of the lake to the surface at each station. Therefore, these samples differed from the long-term samples in that they were from a single tow and were not volume weighted. Preservation and enumeration followed the methods described above. Total annual richness ($S_{1,4,\text{cum}}$) was determined for individual stations, in addition to a composite richness for all 10 stations.

To determine the influence of increasing the number of stations, we calculated cumulative richness for varying numbers of stations for each sampling date. We calculated richness by randomly selecting subsamples comprising from one to 10 stations and calculating a total species list for each subsample. This randomization procedure was repeated 1000 times to determine a mean cumulative richness and standard deviation.

Multiple samples within a year

In the entire database, there were nine occasions when a lake was sampled more than 20 times within a year. We used these data to investigate how increases in the number of intraannual samples influence the estimated species richness. Annual species richness ($S_{1,i,\text{cum}}$) was estimated for each lake–year by subsampling the database, without replacement, 1–20 times. We drew one sample from every month before a second from any particular month could be drawn to prevent temporal clumping. We calculated species richness as the total number of species in the subsampled database. This procedure was repeated 1000 times to obtain a mean richness estimate and standard deviation.

We used the 1980 database to compare the performance of three indices of species richness: cumulative, asymptotic richness, and Chao's index. In 1980, each lake was sampled at least 17 times. Cumulative species richness (S_{cum}) was the total species pool for a particular number of samples. Cumulative species richness for different numbers of samples from one to 17 was obtained by repeating this analysis for all possible combinations.

The asymptotic richness (S_{asym}), based on a Walford plot (Ricker 1975), was obtained by calculating where mean cumulative richness (S_{cum}) at subsample size t versus mean cumulative richness at subsample size $t + 1$ intersects the 1:1 line.

Chao's index provides a relatively unbiased nonparametric estimate of total species richness (Colwell and Coddington 1994) and has been used in other studies to estimate zooplankton richness (Dumont and Segers 1996). The number of species in the lake, S^*, was estimated based on the occurrence of rare species according to

$$S^* = S_{\text{obs}} + (L^2/2M)$$

where L is the number of species that occurred in only one sample, M is the number of species that occurred in exactly two samples, and S_{obs} is the observed richness (Chao 1984). We estimated Chao's richness (S_{chao}) using subsample sizes ranging from 2 to 17.

Multiple-year richness

To estimate long-term species richness, we used cumulative species richness (S_{cum}), Chao's index (S_{chao}), and asymptotic richness (S_{asym}). Individual subsamples, however, were based on annual richness estimates obtained from six monthly samples ($S_{1,6,\text{cum}}$). We used six samples because this was the largest number of samples consistently taken throughout the entire database.

Comparison of richness estimates

We compared richness ranking among lakes obtained for single-year and multiple-year richness estimates. Spearman rank correlations were calculated, comparing each of the three methods for estimating richness (cumulative, asymptotic, and Chao's Index), single-year and multiple-year richness, and 1, 6, and 12 subsamples.

In an attempt to identify why correlation of single-year and multiple-year richness was low, we calculated the Spearman rank correlation coefficient for asymptotic ($S_{12,6,\text{asym}}$) and mean annual species richness ($S_{1,6,\text{asym}}$) in all eight lakes under several different scenarios; (*i*) we removed singlets from the database (species that occurred in only one year), (*ii*) typical littoral species were removed, (*iii*) rare species (with a probability of detection, $P < 0.25$) were removed:

$$P = 1 - (1 + m/k)^{-k},$$

where P is the probability of detecting at least one individual assuming that species approximated a negative binomial distribution across time, m is the number of individuals in the sample, and k is a parameter that is estimated as $k = x^2/(s^2 - x)$, where x is the sample mean density and s^2 is the sample variance.

Gradients in richness estimates are frequently compared with environmental gradients to determine factors controlling community structure (pH: Yan et al. 1996; habitat area: Browne 1981 and Yan et al. 1996; productivity: Dodson 1992; water hardness: Carter et al. 1980). However, the relationship between environmental variables and richness may be influenced by the method and temporal scale used to estimate richness. We compared several explanatory variables for both single-year and multiple-year richness estimates. The importance of log area, maximum depth, conductivity, pH, total phosphorus, and flushing rate in accounting for variation in richness among lakes was evaluated using a forward, stepwise multiple regression. None of these variables were significantly correlated with each other, using a Bonferroni adjustment for multiple comparisons (Table 2).

Results

Estimation of single-year richness

Multiple stations within Plastic Lake

Annual richness ($S_{1,4,\text{cum}}$) was higher at the central station (16 species) than at all other stations (12–15 species, mean = 12.8). This occurred probably in part because the central station is deeper, and therefore the sample represents a greater volume of water and also includes hypolimnetic habitat not occurring at some of the shallower stations. We found that the

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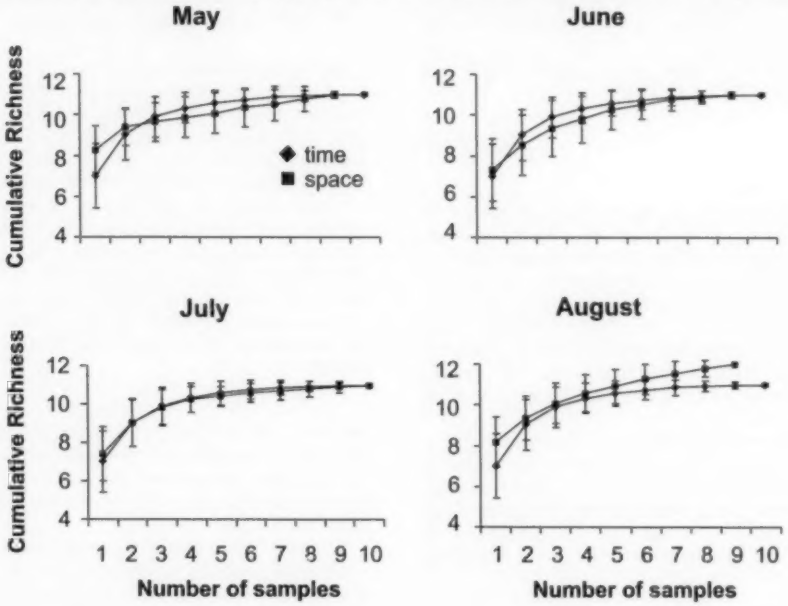
Table 2. Pearson correlation coefficients for explanatory variables of richness.

	log area	Max. depth	Conductivity	pH	Total phosphorus	Flushing rate
log area	1					
Max. depth	0.34	1				
Conductivity	0.60	0.70	1			
pH	0.28	0.43	0.60	1		
Total phosphorus	0.43	–0.21	0.00	–0.61	1	
Flushing rate	0.10	0.20	0.14	0.71	–0.56	1

Note: None of the variables was significantly correlated with each other, using a Bonferroni adjustment for multiple comparisons.

Fig. 1. Mean cumulative richness for two sampling regimes in Plastic Lake. Spatial samples (squares) were taken in May, June, July, and August at 9 or 10 locations in Plastic Lake in 1985. Intraannual samples (diamonds) were taken during the ice-free season in 1985. Both richness estimates were obtained by repeatedly subsampling the database for different combinations of the same numbers of samples.



number of species detected increased with depth sampled ($P = 0.001$, $R^2 = 0.29$), although there was considerable variation in the relationship. The central station, however, underestimated the total species pool, based on the cumulative richness of all stations, across all months (16 species at the central station versus 21 total species at all stations). Four of the five species that were not detected at the central station were detected at only one station on one date and typically occurred at low densities (<0.12/L).

Increases in the temporal extent of sampling (more samples within a year) and increases in spatial extent (more sampling stations) resulted in higher cumulative richness (Fig. 1). Cumulative richness was similar for a given number of samples for both sampling methods, except that in August, spatial samples resulted in slightly higher cumulative richness estimates. For May, June, and July, cumulative species richness was the same after nine samples had been taken.

Multiple samples within a single year

A single sample ($S_{1,1}$), randomly taken during the ice-free season, resulted in the detection of less than 50% of the total species pool obtained from 20 samples ($S_{1,20,cum}$) taken throughout the ice-free season (mean = 48.6% ± 9.9 SD). Species richness of single samples taken in 1980 ranged from 8 to 13 species in the eight lakes (Table 3). The coefficient of variation among sample dates ranged from 14 to 26% and averaged 16%. Species richness ($S_{1,1}$), estimated from individual samples was compared among months to determine if any month provided a best estimate of annual species richness. Although there was no significant difference in the percent species detected each month (ANOVA, $P = 0.36$), the greatest number of species was detected in June (55% of total richness based on 20 samples) and the least was detected in November (39%) (Fig. 2).

More intraannual samples were required as the desired level of species detection increased (Fig. 3). The number of samples required to detect between 50 and 90% of species present in a year increased rapidly from two to 10. To detect 80% of the total species pool, at least five samples (± 2) needed to be examined (Fig. 3). This was approximately equivalent to

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Table 3. Mean species richness for each of the Dorset Lakes, estimated at several temporal scales.

	Number of samples										
	Single-year richness						Multiple-year richness				
	1	3	6	9	12	15	1	3	6	9	12
Cumulative											
Blue Chalk	9.6	14.9	18.0	19.2	20.5	21.1	15.3	18.2	20.4	22.8	23.0
Chub	9.0	14.0	17.1	19.0	20.0	21.0	15.0	18.1	20.4	22.8	26.0
Crosson	10.1	13.1	14.6	15.4	16.6	17.5	15.3	18.9	21.6	22.8	24.0
Dickie	9.5	13.4	15.6	16.7	17.7	18.5	16.3	20.8	24.4	27.3	31.0
Harp	11.0	14.9	17.3	19.4	20.8	22.1	16.0	18.4	21.1	22.8	25.0
Heney	8.1	10.8	13.1	13.9	14.6	15.2	11.4	16.6	22.8	26.0	
Plastic	8.1	11.4	12.9	13.5	14.1	14.7	12.3	15.4	18.0	19.5	
Red Chalk	12.3	17.8	19.6	20.3	21.1	21.8	17.3	20.2	22.3	23.5	24.0
Mean	9.7	13.8	16.0	17.2	18.2	19.0	14.8	18.3	21.4	23.4	25.5
Asymptotic											
Blue Chalk		16.4	18.6	19.4	20.3	20.8		22.7	22.2	26.1	24.1
Chub		15.8	18.1	19.4	20.1	20.8		19.4	21.6	25.1	40.5
Crosson		13.7	14.7	15.4	16.3	17.3		32.7	23.7	23.7	24.4
Dickie		14.2	16.1	16.7	17.5	18.2		23.4	26.9	29.9	38.2
Harp		18.4	18.6	20.7	21.8	23.0		19.5	25.6	25.5	30.5
Heney		11.9	15.0	14.7	15.0	15.3		27.7	42.9	32.7	
Plastic		12.3	13.1	13.5	13.8	14.3		18.3	20.6	21.1	
Red Chalk		19.0	19.8	20.1	20.5	21.0		22.8	23.6	24.3	24.4
Mean		15.2	16.7	17.5	18.2	18.8		23.3	25.9	26.0	30.3
Chao's index											
Blue Chalk		20.1	21.9	26.9	31.1	29.3		26.0	26.0	32.0	25.3
Chub		17.3	21.4	22.8	29.3	28.0		22.5	24.5	32.9	44.0
Crosson		16.2	19.5	17.9	20.3	18.3		26.7	27.5	24.5	26.3
Dickie		17.7	22.6	21.3	24.4	32.9		24.5	30.3	33.6	34.6
Harp		17.7	19.9	22.7	33.2	35.3		21.2	26.7	28.3	27.7
Heney		10.7	13.7	18.6	22.3	20.2		34.0	45.6	46.2	
Plastic		17.7	19.9	22.7	33.2	35.3		20.1	22.0	22.0	
Red Chalk		21.2	24.0	23.6	24.7	24.5		23.0	25.1	26.6	26.0
Mean		17.3	20.4	22.1	27.3	28.0		24.8	28.5	30.8	30.6

Note: Three methods of estimation were used: cumulative, asymptotic, and Chao's index. Single-year richness was calculated using 1980 data.

Fig. 2. Mean monthly richness of Dorset Lakes, based on single samples taken in 1980. Vertical bars represent the SE of the mean. $S_{1,6}$ indicates the mean single-year richness, based on six samples per year.

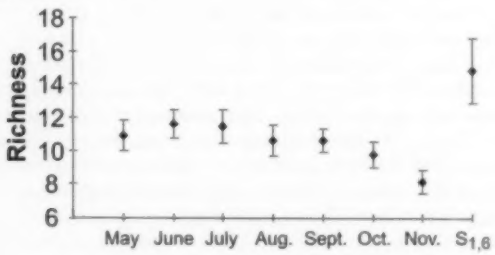
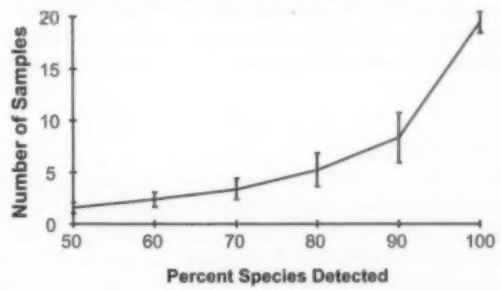


Fig. 3. Number of samples required per year to detect various percentages of the total richness, based on 20 samples per year ($S_{1,20,cum}$). Values represent averages based on nine lake–year combinations. Vertical bars represent the SE of the mean.



monthly sampling during the ice-free season. To detect 95% of the species, 13 samples (± 3) were required (Fig. 3). This required a biweekly sampling regime during the ice-free season.

Richness estimates derived from Chao's index were always greater than estimates obtained using cumulative and asymptotic methods (Table 3; Fig. 4). In general, cumulative and asymptotic richness estimates were similar, although cumulative richness was frequently higher when 12 or more samples were

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Table 4. Species list of crustacean zooplankton from the Dorset study lakes.

Species	Blue Chalk	Chub	Crosson	Dickie	Harp	Heney	Plastic	Red Chalk
Acroperus harpae				8		11		
Alona guttata	8	8	8	17		22		
Bosmina longirostris	100	92	100	100	100	100	100	100
Ceriodaphnia lacustris		8		8		22	20	8
Chydorus bicornutus				8				
Chydorus sphaericus	50	17	58	33	100	44	10	17
Daphnia ambigua	8	92	33	83	17		90	25
Daphnia catawba	33	50		67	8	11	10	25
Daphnia dubia	100	8	17	25	8			92
Daphnia galeata mendotae	100	67	100	67	100	44	20	100
Daphnia longiremis		100	100	17	8	11	40	100
Daphnia pulex	92	8	8	25			40	92
Daphnia retrocurva	25	100	33	100	92	100		42
Eubosmina coregoni	17		25	17		11		
Eubosmina tubicen	42	75	75	100	100	100	100	100
Holopedium gibberum	100	100	100	100	100	89	100	100
Leptodora kindtii	33	33	33	50	25			42
Ophryoxus gracilis		8						
Polyphemus pediculus		25		8	33	11	80	25
Sida crystallina			17	8	8	33		100
Chydorus globosus						11		
Diaphanosoma spp.	100	100	100	100	100	100	100	100
Lepto diaptomus ashlandi						11		
Lepto diaptomus minutus	100	100	100	100	100	100	100	100
Skisto diaptomus oregonensis	100	8	100	17	33		10	100
Lepto diaptomus sicilis			8		92			
Epischura lacustris	100	92	83	75	92	44	30	50
Senecella calanoides				8	100	11		
Cyclops bicuspidatus thomasi	100	42	100	75	100	22	50	100
Cyclops scutifer	100	100	100	100	17	11	100	100
Cyclops vernalis	17	25	25	50	58			
Eucyclops agilis				17		11		
Eucyclops speratus	8							8
Mesocyclops edax	100	100	100	100	92	100	100	100
Orthocyclops modestus		42		42	17	11	40	
Tropocyclops prasinus mexicanus	92	100	100	100	100	100	90	100

Note: Numbers indicate the percentage of years species were present in each of the eight lakes.

taken and asymptotic richness tended to be higher when fewer than nine samples were taken. Variation among asymptotic richness estimates based on different numbers of samples was low (mean = 7%). In contrast, the coefficient of variation among estimates of Chao's richness for different numbers of samples was highest (Chao mean = 23%, cumulative mean = 16%).

Multiple-year richness

There was considerable temporal variation in the presence of species from year to year (Table 4). A single-year sample, based on six samples per year ($S_{1,6,cum}$), predicted 60% of the total observed species pool for 12 years ($S_{12,6,cum}$). Although annual richness underestimated the long-term richness, it was consistent from year to year (mean coefficient of variation across years = 13%).

For each richness index used, richness estimates were highly dependent on the number of samples taken (Fig. 4). Chao's index almost always predicted a higher richness than the other two indices, regardless of sample size. Chao's estimates increased the most between one and four years. When more than four years of samples were used to estimate richness, variation in richness estimates was much smaller; the coefficient of variation among number of years sampled was reduced from 21% when all samples were included to 9% when the number of years was greater than four Asymptotic richness was always higher than cumulative richness estimates. The coefficient of variation among richness estimates obtained from different numbers of samples was highest for Chao's index (mean of lakes = 21%), intermediate for cumulative (mean of lakes = 15%), and lowest for asymptotic (mean of lakes = 12%). Variation in richness estimates was generally highest for Heney Lake. This results from the occurrence of a large number of episodic species that appeared in only one year.

Correlation among richness estimates

Spearman rank correlation of richness estimates of lakes

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Table 5. Spearman rank correlation matrix of single-year and multiple-year richness estimates.

	Single-year samples								Multiple-year samples							
	Cumulative			Asymptotic		Chao's			Cumulative			Asymptotic		Chao's		
	1	6	12	6	12	1	6	12	1	6	12	6	12	1	6	12
Cu 1, 1	1	0.49	0.60	0.55	0.54	0.99	0.09	0.09	0.64	0.32	–0.49	0.26	–0.55	0.64	0.03	–0.60
Cu 1, 6		1	0.94	0.99	0.83	0.41	0.60	0.60	0.38	–0.20	–0.46	–0.37	–0.41	0.38	–0.60	–0.54
Cu 1, 12			1	0.99	0.94	0.55	0.49	0.66	0.46	–0.12	–0.29	–0.2	–0.23	0.46	–0.54	–0.37
As 1, 6				1	0.90	0.49	0.55	0.64	0.43	–0.16	–0.38	–0.29	–0.32	0.43	–0.58	–0.46
As 1, 12					1	0.49	0.26	0.83	0.35	–0.23	–0.20	–0.09	–0.14	0.35	–0.43	–0.26
Ch 1, 1						1	0.12	0	0.72	0.46	–0.35	0.38	–0.44	0.72	0.12	–0.49
Ch 1, 6							1	–0.03	0.64	0.41	0.06	–0.03	–0.06	0.64	–0.20	–0.20
Ch 1, 12								1	–0.14	–0.64	–0.14	–0.26	–0.03	–0.14	–0.43	–0.09
Cu 1, 6									1	0.81	0.07	0.61	–0.16	1.00	0.32	–0.26
Cu 6, 6										1	0.35	0.75	0.09	0.81	0.61	0.06
Cu 12, 6											1	0.41	0.94	0.07	0.23	0.93
As 6, 6												1	0.12	0.61	0.89	0.14
As 12, 6													1	–0.16	–0.06	0.99
Ch 1, 6														1	0.32	–0.26
Ch 6, 6															1	0.03
Ch 12, 6																1

Note: Cumulative, asymptotic, and Chao's estimates are presented. For the single-year samples, estimates are based on annual estimates using 1, 6, or 12 samples within each year. For multiple-year estimates, estimates are based on six seasonal samples and 1, 6, or 12 annual samples. Cu, cumulative richness; As, asymptotic richness; Ch, Chao's estimate of richness. The first number after the prefix Cu, As, or Ch indicates the number of years on which the richness estimate is based; the second number indicates the number of samples within a year.

among single-year sampling regimes was high. That is, estimates based on single years were similar regardless of the particular sampling regime used (Table 5). Similarly, multiple-year species richness estimates, regardless of regime, were similar to each other. In contrast, correlations between single-year and multiple-year estimates were extremely low and frequently negative. That is, the number of species found in a single year was not a good predictor of the number of species found in multiple years. In general, the correlations between richness rankings were highest when numbers of samples were equal, regardless of the index type (mean $r_s = 0.87$ for comparisons between multiple-year samples and mean $r_s = 0.79$ for comparisons between single-year samples). When comparisons were made using the same index, but different numbers of samples, correlation was low (mean $r_s = 0.43$ between single-year samples, mean $r_s = 0.21$ between multiple-year samples, and mean $r_s = -0.03$ between single- and multiple-year samples). Single-year richness estimates (e.g., $S_{1,1}$) did not correlate with long-term estimates (e.g., $S_{12,6,cum}$, $S_{12,6,asym}$, or $S_{12,6,chao}$) ($r_s = -0.49$, –0.55, and –0.60, respectively). Correlation coefficients between $S_{12,6,asym}$ and $S_{1,6,asym}$ improved when rare species were removed. The greatest improvement was obtained when singlets (species that occur in single years) were removed ($r_s = 0.04$ when all species were included, 0.97 when singlets were removed, –0.06 when littoral species were removed, and 0.36 when species with less than a 0.25 probability of detection were removed). When episodic species were removed from the analyses, single-sample richness was a good predictor of the long-term ranked richness among lakes.

Explanatory variables of richness

The relationship between species richness and environmental variables was dependent on both the index type and the number of years used to estimate richness. Depth explained 61% of the variation among lakes in single-year estimates of richness ($S_{1,6,cum}$). Lake flushing rate, log area, conductivity, total phosphorus, and pH did not contribute to the variation in richness among lakes. When other indices were used to calculate richness, different results were obtained. Conductivity explained 65% of the variation in single-year asymptotic richness ($S_{1,6,asym}$) ($P = 0.02$) and none of the environmental variables explained variation in Chao's single-year richness ($S_{1,6,chao}$). Variation in multiple-year richness was explained by total phosphorus when either cumulative or asymptotic richness was estimated ($S_{12,6,cum}$: $P = 0.07$, $R^2 = 0.45$; $S_{12,6,asym}$: $P = 0.095$, $R^2 = 0.40$). None of the environmental variables explained a significant amount of the variation in richness estimated using Chao's index ($S_{12,6,chao}$).

Discussion

Our estimates of total species richness, based on an extensive 12-year database, were considerably higher than expected from two previous multilake surveys. Dodson (1992) predicted that between eight and 10 planktonic crustacean species should be found in lakes within the size range of the Dorset lakes. Similarly, Patalas (1990) suggested that lakes in northwestern Ontario would have, on average, 10.7 species. We estimated the Dorset lakes to have mean annual richness from 11.4 to 17.3 species and total species pools ranging from 21 to 40 species, based on multiple-year asymptotic richness (Table 3). Our values are higher than Dodson's and Patalas' estimates for two reasons: our estimates were based on more samples, collected over an extended period (six monthly samples for 8–12 years) and we did not eliminate typically littoral species that were caught in our pelagic samples (as in Dodson's study). The lakes for both Patalas' and Dodson's studies were sampled infrequently; single samples in Patalas' study and a minimum

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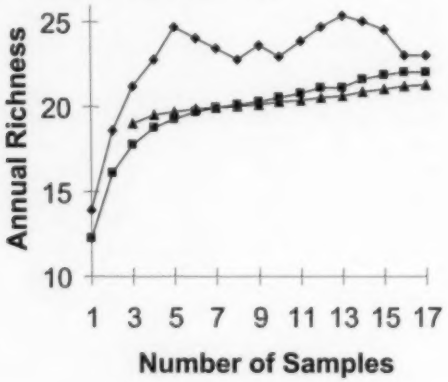
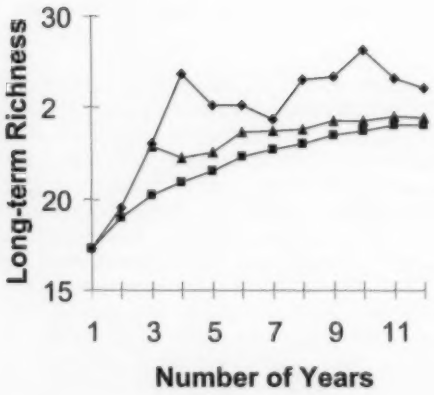
Fig. 4. Relationship between the number of samples taken and the single-year richness (top panel) and the multiple-year richness (bottom panel) for Blue Chalk Lake. Single-year richness was based on samples taken in 1980. Richness was estimated using three different methods: cumulative (squares), asymptotic richness (triangles), and Chao's index (diamonds).





of three samples in Dodson's study. When single sample surveys were used to estimate species richness in Dorset area lakes, results similar to Dodson's and Patalas' estimates were obtained. Yan and Strus (1980) found 9.3–14.6 species per sample on average in Blue Chalk, Jerry, Chub, Harp, and Dickie lakes in 1977. Keller and Yan (1991) reported a mean of 10 species per sample in 24 nonacidic (pH > 6.0) Dorset area lakes. Therefore, with comparable sampling effort, our richness estimates for the Dorset lakes are similar to previous studies. However, our richness estimates based on long-term data suggest that it is likely that all of these investigators underestimated the actual size of the species pool. It is interesting to note that our richness estimates based on many samples in a single lake approach Patalas' (1990) estimates for the number of taxa in many lakes in a region of Canada. One possible explanation is that most species within a region may be present in each lake at some point in time, although they are very rare.

Our comparison of spatial and seasonal sampling in Plastic Lake suggests that increasing the temporal or spatial extent of sampling improves the richness estimate. The identity of the species, however, varied depending on sampling regime. *Eubosmina tubicen*, *Holopedium gibberum*, *Leptodiaptomus minutus*, and *Daphnia ambigua* were detected only in the temporal samples, and *Alona* sp., *Chydorus sphaericus*, *Daphnia retrocurva*, and *Eurycercus lamellatus* were collected only in the spatial samples. Therefore, the decision to choose one sampling regime over another depends on the goals of the study. Although species richness estimates were similar, multistation samples tended to detect more littoral species whereas the multidate samples tended to detect typically pelagic species with temporally variable abundances. This result is probably dependent on lake size. Patalas and Salki (1993) found that the proportion of total species detected at a single sampling station was dependent on lake size, with a higher proportion of the total species being detected in smaller lakes. Similarly, fewer spatial samples were required in small lakes (<170 ha) to capture 90% of the species, although Lake 373 (27 ha) was a notable exception to this trend. Therefore, the value of spatial versus temporal sampling may depend on the size of the lake. All of the Dorset lakes used in our study are small, ranging from 20 to 100 ha in size. Had we used a larger lake than Plastic Lake, we may have found a higher importance of spatial samples relative to temporal samples.

Mean annual richness did not predict richness based on multiple-year samples. This result was not surprising, as Hurlbert (1971) suggested it as a general possibility in his review of species diversity measures. In our study lakes, as the temporal extent of sampling increased, total richness also increased, probably because of increased sampling effort (i.e., identifying a larger number of individuals) and because of temporal oscillations in species abundances. Zooplankton undergo both seasonal shifts in population abundances (Sommer et al. 1986) and interannual changes in species composition (Browne 1981). Single samples or single-year samples failed to detect highly variable species that were present infrequently throughout the entire sampling record. Correlations between single-year and multiple-year richness were low and almost always negative. Some lakes with low species richness in single-year estimates had high multiple-year richness. For example, Heney Lake was tied for the fewest species when richness was assessed on an annual basis. When richness was determined by extrapolation from long-term data, however, Heney Lake had the most species. Heney Lake is unique in that it has a high species turnover rate; approximately 25% of the species in Heney Lake changed from year to year (S.E. Arnott, unpublished data). Mean annual richness remained relatively constant, but the total species pool increased annually. In contrast with this, however, other lakes maintained their relative ranking for both short-term and long-term richness estimates. Blue Chalk Lake had both a low short-term richness ($S_{1,1}$) and a low richness based on long-term estimates ($S_{9,6,cum}$). This discrepancy arises, in part, because in different lakes, species may be appearing and disappearing from year to year at different rates. The difference between mean annual species richness ($S_{1,6,cum}$) and multiple-year richness ($S_{12,6,cum}$) was highly correlated with measured species turnover (S.E. Arnott, unpublished data) (linear correlation: $r = 0.868$, $P = 0.005$).

Much of the difference between short-term and long-term richness arises, in part, from species appearing for single years.

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When singlets were removed, the correlation between mean annual and long-term richness improved. Most of the singlet species occurred at relatively low densities (mean = 0.014 individuals/L ± 0.018 SD) compared with the overall mean density of species (mean = 0.489 individuals/L ± 1.187 SD) and it is uncertain what role they play in the community. They may be opportunistic species that occasionally arise when certain environmental conditions are met, or they may be species that are always present but are undetectable using typical sampling and counting regimes. These rare species may function as "ecological memory," enabling the community to rapidly respond to environmental changes (Padisak 1992). Therefore, the decision to use single-year richness versus long-term richness should be at least partially based on the importance of rare, episodic species to the hypotheses being tested. If rare species are removed from the analyses, there is good correlation between single-year richness and multiple-year richness, indicating that single-year estimates may provide useful estimates of the species pool.

As a result of these temporally dependent switches in richness rankings, there were differences in how short-term richness and long-term richness correlated with environmental variables. There were also differences in correlations, depending on indices used for richness estimates. Depth was an important explanatory variable for cumulative annual richness ($S_{1,6,cum}$) and conductivity explained some of the variance in asymptotic annual richness ($S_{1,6,asym}$). For long-term richness estimates ($S_{12,6,cum}$ and $S_{12,6,asym}$), total phosphorus was the only significant explanatory variable. In addition to the influence of study duration and richness measure, these results are also dependent on the number of lakes in the analysis. Using a larger set of lakes (47) located in the same region as our eight study lakes, Yan et al. (1996) found that single-year richness estimates were correlated with lake acidity and size. Despite our small sample size (eight lakes), the discrepancy among richness estimates derived from a different method suggests that care must be taken when drawing conclusions from species richness data. Magnuson et al. (1994) drew a similar conclusion based on analyses of fish species richness in seven Wisconsin lakes. The coefficient of the species–area relationship was dependent on whether mean annual richness or cumulative richness was used in their paper. As with Magnuson et al. (1994), we conclude that richness determined by sampling should be treated as an "index" rather than total richness. Thus, care must be taken to use standardized sampling efforts and estimators, especially across time because duration of the study influences our perception of the relationship between species richness and explanatory environmental variables.

Annual richness and multiple-year richness estimates provide different information regarding the zooplankton community. Generally, richness estimates based on a single sample represent the number of potentially interacting species in a lake or the number of co-occurring species. It is possible that some of the species may have little or no effect on each other, but there is potential for direct or indirect interactions. It is also possible that species that do not overlap in time may also influence each other through events such as delayed changes in resource quality or supply. However, as a first cut, annual richness may be a good representation of the number of species with the potential for interaction. The annual richness measure may be useful when quantifying the effect of a change in chemistry or the influence of an invading species on community structure. Because the probability of capture is related to abundance, rare species will probably be overlooked. However, it may be justifiable to assume that rare species play a minor role in food web interactions at a given moment in time. Mean annual richness based on several samples throughout the season increases the probability of detecting rare species and also facilitates the detection of seasonally dynamic species. Multiple-year richness represents a longer time scale and takes into account shifts in species composition. It is a measure of the potential species pool. This type of information may be useful in biodiversity studies where researchers are investigating the relationship between richness and stability or resilience. Knowing the total species pool could provide an indication of the potential for functional complimentarity (sensu Frost et al. 1995) within the community. Two habitats with the same mean annual species pool may have very different long-term richness estimates, which could lead to differences in stability. For example, in both Blue Chalk and Chub lakes, we detected approximately 15 species each year ($S_{1,6,cum}$). The asymptotic richness (long-term richness) was much greater for Chub Lake than for Blue Chalk Lake (40 versus 24 species). Because Chub Lake has a large species pool, we might expect that it would be more resistant to disturbance through functional compensation of species (e.g., Frost et al. 1995). That is, species that are negatively influenced by a stress may be replaced by more resistant species with complimentary community functions.

Recommendations

There are many factors that influence the decision to choose one sampling and counting routine over another. It will depend not only on funding resources available, but also on the objectives of the study. For some questions, assessing the number of interacting species at a given point in time is of primary concern. For example, single-sample or single-year estimates may be adequate for determining the relationship between diversity and primary productivity or predator density. Presumably, these factors would be important drivers determining the number of interacting species, and therefore, their influence should be detected at a temporally restricted scale. In contrast, multiple-year richness estimates would be more important in determining differences among lakes in their potential response to stress. Lakes with large species pools, either as rare "memory" species (sensu Padisak) or as temporarily dormant resting stages, may be more resistant to perturbations. In this case, it would be important to extend the sampling schedule for as long as possible. However, in either situation, there is the underlying importance of standardized sampling regimes. Because richness estimates were influenced by the number of both intraannual and interannual samples, methodology should, at least, be consistent among lakes within a survey.

Differences in the method used to predict richness are also worth consideration. All three indices, cumulative, asymptotic, and Chao's, were dependent on the number of samples taken. However, the asymptotic method of estimating richness provided both an estimate of the potential species pool and consistent values regardless of the number of years sampled. Therefore, we recommend use of the asymptotic method of richness estimation for multiple-sample studies.

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Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Variation among individuals in specific growth rate (SGR), feeding, and two measures of swimming performance and their possible interrelationships were investigated in juvenile rainbow trout (*Oncorhynchus mykiss*) kept in groups on either satiation or half-satiation rations. Maximum sustainable velocity (U_{crit}) was measured as an index of aerobic swimming performance and stamina (fatigue time in a fixed-velocity test at 6 body lengths·s^{-1}) as an index of anaerobic performance. Individual performance in both of these tests was found to be significantly repeatable. Trout fed on half-satiation ration exhibited significantly lower mean values of SGR and body size and higher levels of aggression-related fin damage, but no significant differences in stamina, relative or absolute U_{crit}, glycogen content, or plasma cortisol. However, in these fish, there was a significant negative relationship between SGR and relative U_{crit}, a significant positive relationship between SGR and stamina, and a significant positive relationship between SGR and total daily meal. None of these relationships were seen in fish fed to satiation. Plasma cortisol and tissue glycogen stores were not related to SGR. These results indicate that under the intensified competition of restricted ration, there are trade-offs between growth, feeding, and different types of swimming performance.

Résumé : La variation entre individus du taux de croissance spécifique (TCS), de l'alimentation et de deux mesures de la performance natatoire, de même que les relations possibles entre ces paramètres, ont été étudiées chez des truites arc-en-ciel (*Oncorhynchus mykiss*) juvéniles réparties en deux groupes : un premier groupe nourri à satiété et un deuxième groupe à moitié rassasié. La vitesse soutenable maximale (U_{crit}) a été mesurée à titre d'indice de la performance natatoire aérobie, et l'endurance (temps de fatigue dans un test à vitesse fixe de 6 longueurs de corps·s^{-1}) à titre d'indice de la performance anaérobie. On a observé que la performance individuelle dans chacun de ces tests pouvait de façon significative être répétée. Chez les truites à moitié rassasiées, on a observé des valeurs moyennes de TCS et de taille corporelle significativement inférieures, et des dommages aux nageoires liés à l'agression significativement plus importants, mais aucune différence significative en ce qui a trait à l'endurance, à l'U_{crit} relative ou absolue, à la teneur en glycogène et au cortisol plasmatique. Cependant, il existait chez ces poissons une relation négative significative entre le TCS et l'U_{crit} relative, une relation positive significative entre le TCS et l'endurance, et une relation positive significative entre le TCS et la ration alimentaire totale quotidienne. Aucune de ces relations n'a été observée chez les poissons nourris à satiété. Il n'y avait pas non plus de relation entre, d'une part, le cortisol plasmatique et le glycogène tissulaire et, d'autre part, le TCS. Ces résultats montrent que l'intensification de la compétition liée à la limitation de la ration alimentaire induit des compromis entre la croissance, l'alimentation et différents types de performance natatoire.

[Traduit par la Rédaction]

Introduction

When groups of fish are raised under continuous swimming conditions, they show both faster growth and improved swimming performance (Davison 1997). The faster growth is thought to be due to an increase in the ratio of protein synthesis to protein degradation (Houlihan and Laurent 1987) and a reduction of nonspecific aggressive activity associated with continuous exercise, as well as the implementation of energy-efficient "ram ventilation" (Christiansen and Jobling 1990). However, in themselves, growth rate and swimming ability had been viewed as unrelated until very recently, and very few studies have been conducted that investigate any possible interactions between them within individual animals. Furthermore, many physiologists have traditionally viewed individual variation as no more than "statistical noise," which could be avoided or masked with the use of a sufficiently large number of animals. More recently, individual variation in swimming performance has been shown to be repeatable in largemouth bass (*Micropterus salmoides*) (Kolok 1992), altering its status from a mere statistical annoyance to something of real importance worthy of study. By experimentally exploiting, rather than suppressing, the existence of individual differences in growth rate and swimming ability, it is possible to investigate

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potential trade-offs between these two aspects of trout life history. A pioneering recent study on fathead minnow (*Pimephales promelas*) suggests that there may indeed be a trade-off between growth rate and aerobic swimming ability (Kolok and Oris 1995), but the mechanistic basis of the trade-off was not investigated, nor was the possibility of a similar trade-off with anaerobic swimming ability. To date, there have been no studies of this nature on rainbow trout (*Oncorhynchus mykiss*).

Individual variation in feeding may be an important causal element in the interaction between growth rate and exercise ability. Recent developments in X-ray technology have greatly facilitated the study of individual feeding with a minimum of disturbance to the fish (e.g., Carter et al. 1993; McCarthy et al. 1993). With this approach, it has been shown that position in the feeding hierarchy affects growth, with subordinate individuals receiving less food than dominant ones (McCarthy et al. 1992, 1993). Subordinate fish also have significantly lower food conversion efficiencies and grow more slowly even if fed the same ration as dominant fish (Abbott and Dill 1989). Slower growth rates are almost certainly exacerbated by the increased levels of stress and physical activity among subordinate fish, but this may not represent a complete explanation (Abott and Dill 1989).

The present study employed these approaches, together with swim tunnel tests (Hammer 1995), to examine individual variation in swimming ability, growth rate, and feeding in juvenile rainbow trout. The maximum sustainable swimming speed, or U_{crit} (critical velocity), was used to measure aerobic capacity (Brett 1964) and the fixed-velocity test of fatigue to measure stamina (Brett 1967; Beamish 1978). This latter test has recently been evaluated by McDonald et al. (1998), who concluded that it provided a nondestructive approximation (as opposed to destructive tissue metabolite analysis) of anaerobic capacity. The first specific objective was to examine the relationships between specific growth rate and these two ecologically relevant measures of swimming performance, and a second objective was to relate the relationships between swimming performance and growth rate to feeding and to examine the effects of different rations on individual growth rates.

Materials and methods

Juvenile rainbow trout (series 1: 11–25 g; series 2 and 3: 4–11 g) were obtained from Humber Springs Trout Hatchery in Orangeville, Ont. The fish were kept at 15 ± 1°C in dechlorinated Hamilton, Ont., tap water (see Alsop and Wood 1997) with a photoperiod of 12.5 h light : 11.5 h dark and constant flow-through at a rate of ~1 L·min^{-1}. Holding tanks consisted of 60-L tubs covered by fabric mesh and shaded by opaque plastic lids. Three pieces of ABS piping were added to each tank to act as shelters after preliminary experiments showed a high degree of aggression-related mortality.

Series 1: Repeatability of swim performance

Eight fish (mean initial mass 18.70 ± 1.04 g) were anaesthetized individually in 0.1 g tricaine methanesulfonate (MS 222)·L^{-1} set to a pH of 8.0 with 3.0 M NaOH, weighed (to 0.01 g), measured for fork length (to the nearest 0.1 cm), and marked for individual identification using a PanJet ink injector and Alcian Blue 8GX dye. The fish were then placed in a 60-L holding tank and allowed to recover for 48 h. The fish were offered food 2 days after marking and were swum for the first time 2 days after feeding (and therefore had been fasted for 48 h prior to swimming). On the day of swimming, all eight fish were identified, weighed, measured for fork length, maximum height, and maximum width, and placed in the 50-cm (20-L) swimming chamber of a modified 100-L Beamish-type respirometer calibrated prior to use with a Kent Microflo type 265 propellor-style flowmeter. The fish were allowed to habituate to the tunnel at 1 body length (BL)·s^{-1} (all increments were based on the mean length of the group) for 2 h. After this initial period, the velocity of the water was increased by 0.75 BL·s^{-1} every 40 min. Fish that became impinged against the grating at the rear of the swim chamber were prompted to resume swimming by manual removal from the grating and reintroduction into the current. When a fish could no longer be prompted to swim in this manner, it was judged exhausted and removed. The relative U_{crit} (BL per second) of each fish was then calculated as in Brett (1964). Following this initial trial, the fish were given 8 days to recover and were offered food every 48 h. A second identical U_{crit} trial was carried out at the end of this recovery period (with a 48-h fasting period prior to swimming). The U_{crit} values were not corrected for blocking effects, since the total cross-sectional area of the fish (as calculated in Kolok and Oris 1995) did not occupy more than 10% of the area of the swim tunnel. A shocking grid was not used, in accordance with animal care regulations.

A test of the repeatability of stamina was also conducted. Ten fish of similar size (mean initial mass 13.44 ± 0.34 g) were chosen from a larger tank of fish, measured for fork length (to the nearest 0.1 cm), and swum immediately (one at a time) in the 100-L Beamish-type respirometer described above. Each fish was allowed to habituate to the swim tunnel for 5 min, followed by an increase in velocity of 1 BL·s^{-1} every 30 s until a speed of 6 BL·s^{-1} was reached, at which time a stopwatch was started. When the fish became impinged against the rear grating, the timing clock was stopped, and was restarted only if the fish resumed swimming following manual reintroduction into the current. When the fish could no longer be prompted to swim in this way, it was judged exhausted and removed from the tunnel and the total time swum at 6 BL·s^{-1} recorded. After swimming, each fish was weighed, measured for maximum height and width, marked for individual identification, and then allowed to recover for 48 h before being offered food. Thereafter, the fish were fed on three consecutive days to promote replenishment of glycolytic substrates. The fish were then fasted for 48 h before a second identical trial was conducted. In this trial, the individual trout were swum in a different order from the first trial.

Series 2: Growth rate and swim performance

Fish were weighed to the nearest 0.01 g, measured for fork length (to the nearest 0.1cm), marked for individual identification as above, and then placed alternately in each of the four tanks to avoid any "ease-of-capture" biases in any given tank. Twenty fish were placed in each tank, with no significant differences in mean initial mass between any of the tanks (tank 1, 7.67 ± 0.37 g; tank 2, 7.43 ± 0.38 g; tank 3, 7.75 ± 0.66 g; tank 4, 7.47 ± 0.39 g; all $p > 0.60$). Fish in tanks 2 and 3 were fed to satiation three times per day (10:00, 13:00, 16:00; all times ± 0.5 h) using commercial feed (Ziegler Bros., Inc., Gardners, Pa.). This process involved providing ~5–10 pellets at a time until the fish stopped eating, a process that typically took 10–15 min. The total percent body mass of food consumed was then measured and used to calculate a half-satiation diet for the other two tanks (1 and 4), such that these tanks received one half of the food given to the satiation tanks, in terms of percent body mass. These fish were also fed three times per day, but since the amount to be delivered was predetermined and the fish ate quickly, feeding was typically complete in less than 5 min.

The fish were grown for 3 weeks and were weighed and measured weekly (anaesthesia was deemed unnecessarily stressful and was not used). After the initial growing period, the fish were challenged with one of two types of swimming trial. Fish from two tanks (one satiation, one half-satiation) were tested for U_{crit} whereas the other two were tested for stamina using the methods described in series 1. After

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a 1-week recovery period, during which the feeding regime was maintained, the trials were reversed such that each fish had eventually been tested for both types of swimming performance. In all cases, the fish were fasted for at least 18 h prior to swimming. The U_{crit} was evaluated by swimming fish in groups of 10 or less, with groups formed on the basis of body length (with a maximum range of 1.5 cm). Relative U_{crit} (BL per second) was calculated separately for each fish as in Brett (1964) and was multiplied by the individual body length to give absolute U_{crit} (centimetres per second).

The specific growth rate (SGR) of each fish (percent per day) was calculated as in Kolok and Oris (1995) at the time of swimming based on growth over the total growth period. Days on which the fish were swum were not counted as growing days, since the fish were not fed on these days. In addition to mass and length, the fish were measured for maximum height and width prior to swimming, which permitted calculation of both maximum cross-sectional area (π[(0.5 × maximum height) × (0.5 × maximum width)]) and condition factor (100 × mass × length^{-3}).

Series 3: Feeding and growth rate

Fish were kept in two of the same tanks as used in series 2, under the same conditions, except that the temperature was 14 ± 1°C and the flow-through rate was ~0.4 L·min^{-1}. These small differences were dictated by the need to heat the inflowing water during midwinter. Twenty-five fish were placed in each tank, with no significant differences in mean initial mass between the two (tank 1: 5.55 ± 0.18 g, tank 2: 5.34 ± 0.11 g; $p > 0.32$).

The fish were fed three times per day, with one tank fed to satiation and the other fed a half-satiation ration exactly as in series 2. However, the diet was altered. Commercial fish feed (Ziegler Bros., Inc., Gardners, Pa.) was powdered in a standard kitchen blender, mixed with water, and repelleted using a pasta maker. The food was cut into pellets and dried overnight at 60°C. A second diet was prepared in the same manner, but with the incorporation of 8.5-grade (0.400–0.455 mm) lead glass ballotini beads (Jencons USA, Inc., Bridgeville, Pa.) into the food (6% by mass of dry powdered food).

The fish were grown for 3 weeks and weighed and measured weekly. During this time, they were fed the repelleted food without beads. After this initial growing period, the fish were then fed a single meal consisting of the repelleted food containing the ballotini beads (Carter et al. 1993; McCarthy et al. 1993). The ballotini beads did not affect the palatability of the food, as judged from the total meal size. After at least 1 h, the fish were anaesthetized individually as above and X-rayed using a Faxitron 805 portable X-ray machine (3- to 4-s exposure at 70 kVP) to visualize the number of ballotini beads. All X-raying was completed within 3 h of feeding, and the fish were air exposed for no more than 1 min. The condition of the dorsal and caudal fins was also noted at this time, and each was assigned a numerical score between 1 (worst) and 4 (best). This process was repeated three times, with 1-week recovery periods, such that each fish had been X-rayed once after each of the three daily meals. A sample of 180 food pellets was also X-rayed, and <4% of the pellets were found to be devoid of beads.

The beads located in the digestive tracts of the fish were counted from the X-ray image, and the total number of beads found in all of the fish within each tank was used to calculate the percent meal obtained by each individual fish. The amount of food in grams (wet weight) obtained by each fish for each of the three daily meals was calculated by determining the percentage of beads found within each fish and multiplying this by the known amount of food given to the tank. In only three cases were individual fish seen to regurgitate food, and none of the X-rays showed movement of beads further than one third of the way along the digestive tract, suggesting that none of the beads had been evacuated prior to X-raying.

Coefficient of variation (CV) was calculated for each fish based on the three recorded meals (McCarthy et al. 1992). The total daily meal of each fish was also calculated and was corrected for body mass, since it consisted of values obtained over a 3-week period, during which the mass of the fish changed:

(1) Total daily meal =

$$\sum_{n=1}^{3}\left(\frac{(\%\ \text{meal obtained})_n \times (\text{total meal size})_n}{(\text{body mass at time of meal})_n}\right) \times 100\%.$$

After the completion of all three X-ray exposures, the fish were sacrificed in groups of five or six using 0.6 g MS 222·L^{-1}, a concentration that is lethal within 30 s of immersion, thereby preventing the induction of a cortisol response (Beitinger and McCauley 1990). Individuals were identified and blood samples were taken following caudal severance using standard haematocrit tubes. Bodies were freeze-clamped in liquid nitrogen, and the blood was spun for 2 min at 5000 × *g*. Haematocrits were measured, and then blood plasma was withdrawn and frozen in liquid nitrogen. Whole carcasses and blood plasma were stored at –70°C for several days. Whole-body glycogen was analyzed enzymatically as outlined in Bergmeyer (1965), and plasma cortisol was measured using an ImmuChem Coated Tube Cortisol ^{125}I radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa, Calif.).

Statistical analysis

Statistical analyses were performed using Statistix v1.0 for Windows (© 1996 Analytical Software), SigmaPlot v4.0 for Windows 95 (© 1997 SPSS, Inc.), and Quattro Pro v7.0 for Windows 95 (©1996 Corel Corporation, Ltd.). In all cases, differences in means between groups were evaluated using two-sample *t*-tests, and all differences in variance between groups were evaluated using *F*-tests. Single, multiple, and forward stepwise linear regressions were performed with Statistix v1.0 using least-squares regression, and the repeatability of performance was determined using the Spearman rank correlation test as in Kolok (1992). All averages are reported as mean ± SE (*n*).

Results

Series 1

The mean U_{crit} of the fish was 5.23 ± 0.09 BL·s^{-1} in the first trial and 5.64 ± 0.10 BL·s^{-1} in the second trial, indicating a significant improvement in performance ($p = 0.0079$, $n = 8$). More importantly, the relative performance of these individual fish was repeatable when swum in groups (Spearman rank correlation coefficient, $\rho = 0.8571$, $p = 0.0065$) (Fig. 1A).

The fish used in the stamina tests also showed a significant increase in performance between trials (mean time at 6 BL·s^{-1}; trial 1, 1.30 ± 0.21 min; trial 2, 4.56 ± 0.82 min; $p = 0.0031$, $n = 10$). As for U_{crit}, the stamina of individual fish was repeatable during the two trials ($\rho = 0.6485$, $p = 0.0425$) (Fig. 1B).

Series 2

A significant *negative* regression was found between SGR and relative U_{crit} in the half-satiation group ($r^2 = 0.2004$, $p = 0.0131$) (Fig. 2A). Relative U_{crit} was also found to be significantly negatively correlated with body mass, fork length, and maximum cross-sectional area (all $p < 0.05$) but not with condition factor ($p > 0.27$). A forward stepwise linear regression including all of these variables showed SGR to be the most explanatory model (explaining roughly 20% of the variance in U_{crit} observed). Mass, cross-sectional area, length, and SGR were found to be positively correlated with one another in the half-satiation group (all $p < 0.05$).

Linear regressions were performed using the regression residuals of relative U_{crit} versus SGR. No significant regressions

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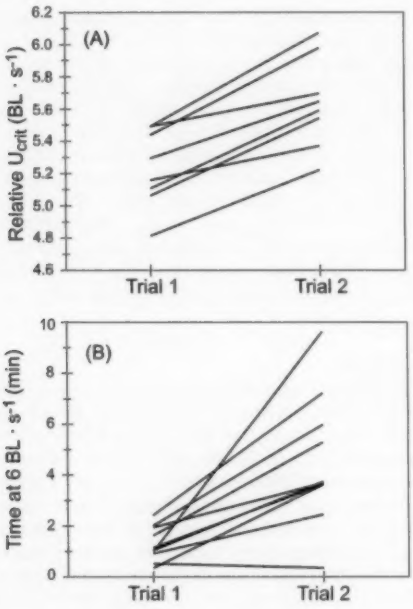
Fig. 1. Repeatability of two different types of individual swimming performance, (A) relative critical swimming velocity (U_{crit}) ($\rho = 0.8571$, $p < 0.01$) and (B) stamina (swimming duration at a fixed velocity) ($\rho = 0.6485$, $p < 0.05$). Both types of swimming showed repeatability and an improvement in performance over the two trials.



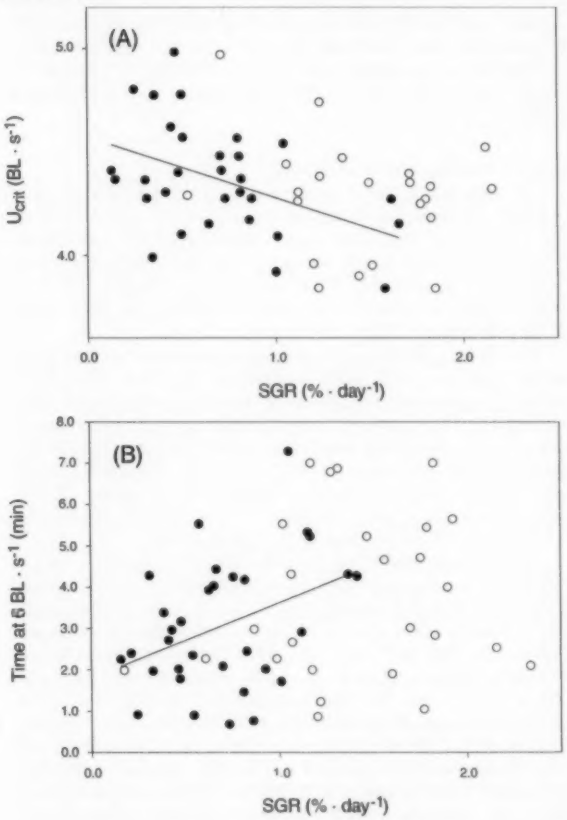
were found between these residuals and mass, length, or cross-sectional area (all $p > 0.19$). A significant regression was found between the regression residuals from the relationship between mass and SGR and those from the relationship between mass and relative U_{crit} ($r^2 = 0.172$, $p < 0.02$).

The fish fed to satiation did not show any significant correlation between relative U_{crit} and SGR or between relative U_{crit} and mass, length, cross-sectional area, or condition factor (all $p > 0.10$). When the data from both diet groups were pooled together, a similar relationship to that found in the half-satiation group alone was observed, with significant negative correlations between relative U_{crit} and SGR, mass, length, and cross-sectional area (all $p < 0.05$).

The fish were also evaluated in terms of absolute U_{crit} (centimetres per second). In both diet groups, significant positive regressions were found between absolute U_{crit} and mass, length, and cross-sectional area (all $p < 0.05$) but not with SGR or condition factor (all $p > 0.43$). No significant differences in mean absolute U_{crit} ($p > 0.50$) or variance ($p > 0.13$) were found between the two diet groups (Table 1).

In contrast with the relationship with relative U_{crit}, a significant *positive* correlation was found between SGR and stamina in the half-satiation group ($r = 0.3848$, $p < 0.03$) (Fig. 2B). No significant correlations were found between stamina and mass, length, cross-sectional area, or condition factor (all $p > 0.25$). In the satiation group, stamina was not significantly correlated with SGR or with any of the other variables examined (all $p > 0.74$). When the data for both diet groups were pooled, stamina and SGR were not significantly correlated ($r = 0.2554$, $p = 0.0530$). Furthermore, no significant correlations were found between stamina and mass, length, cross-sectional area, or condition factor (all $p > 0.15$).

Fig. 2. Relationship between (A) critical swimming velocity (U_{crit}) and specific growth rate (SGR) and (B) stamina (swimming duration at a fixed velocity) and SGR among fish fed on satiation (open circles) and half-satiation (solid circles) rations. A significant negative regression ($r^2 = 0.2004$, $p < 0.02$, $n = 30$) was found between U_{crit} and SGR, and a significant positive regression ($r^2 = 0.1481$, $p < 0.03$, $n = 32$) was found between stamina and SGR in the half-satiation group, but not among fish fed to satiation. The regression lines $y = -0.3x + 4.57$ (Fig. 2A) and $y = 1.83x + 1.79$ (Fig. 2B) represent the relationships found in the half-satiation group only.



The mean values of swimming performance and growth rates were also compared between the two diet groups (Table 1). While the fish fed to satiation exhibited a significantly higher mean SGR than the fish fed to half-satiation ($p < 0.0001$), there was no significant difference in relative U_{crit} or fatigue time between the two treatments (all $p > 0.14$).

Series 3

A significant *positive* regression was found between SGR and total daily meal (percent body mass) in the half-satiation group ($r^2 = 0.5413$, $p < 0.0001$). No significant regressions were found between SGR and total daily meal among fish fed a satiation diet ($p > 0.44$) (Fig. 3). As in series 2, fish fed to

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Table 1. Mean values ± SE (n) of mass, length, condition factor, cross-sectional area, specific growth rate (SGR), and swimming performance in the satiation and half-satiation diet groups of series 2.

Variable	Half-satiation	Satiation	p
Initial mass (g)	7.38±0.24 (40)	7.34±0.25 (40)	>0.99
Final mass (g)	9.91±0.39 (33)	13.24±0.63 (29)	<0.0001*
Initial length (cm)	8.7±0.1 (40)	8.7±0.1 (40)	>0.87
Final length (cm)	9.4±0.1 (33)	9.8±0.1 (29)	<0.04*
Condition factor ($100 \times g \cdot cm^{-3}$)	1.11±0.01 (34)	1.16±0.02 (29)	<0.02*
Cross-sectional area (cm^2)	1.6±0.1 (34)	1.9±0.1 (29)	<0.01*
Final SGR ($\%\cdot day^{-1}$)	0.76±0.05 (33)	1.61±0.08 (29)	<0.0001*
Relative U_{crit} ($BL\cdot s^{-1}$)	4.37±0.05 (30)	4.25±0.05 (25)	>0.14
Absolute U_{crit} ($cm\cdot s^{-1}$)	40.69±0.52 (30)	41.26±0.71 (25)	>0.50
Time at 6 $BL\cdot s^{-1}$ (min)	3.06±0.28 (32)	3.73±0.39 (26)	>0.15

Note: Condition factor and cross-sectional area are calculated as the means of measurements taken on both swimming days. Differences in sample size are due primarily to mortality, although some fish that did not swim during the ramp-up phase or that escaped from the swim tunnel have been omitted from the data set.

*Significant difference ($p < 0.05$).

satiation showed significantly higher mean final mass and SGR than those fed a half-satiation ration (all $p < 0.0001$) (Table 2). However, there was no significant difference in mean CV between the two diet groups ($p > 0.80$) (Table 2). Neither of these diet groups showed significant regressions between CV and SGR (all $p > 0.48$) or CV and initial mass (all $p > 0.09$). Fin conditions were evaluated by tabulating the total fin score of each fish using the results of all three evaluation periods and adding the separate scores assigned to the dorsal and caudal fins. No significant correlations were found between SGR and fin score in either of the diet groups (all $p > 0.27$). However, fish in the satiation group showed significantly higher mean fin scores (i.e., less damage) than those in the half-satiation group ($p < 0.006$). No significant correlations were found between either SGR or CV and plasma cortisol level in either diet group (all $p > 0.68$), and no significant differences in mean plasma cortisol levels were found between the two diet groups ($p > 0.34$) (Table 2). Plasma cortisol levels did not increase with sampling order in either of the tanks, indicating that the sampling procedures did not induce a cortisol response.

There were no significant correlations between SGR and whole-body glycogen levels in either diet group, or when the data from both groups were pooled (all $p > 0.23$). In addition, there was no significant difference in mean glycogen levels between the fish kept in the two groups ($p > 0.76$) (Table 2).

When the data from both diet groups were pooled, a weak but significant negative regression was found between haematocrit and SGR ($r^2 = 0.1105$, $p < 0.04$), but this was not seen in the individual groups. Fish in the half-satiation diet group showed a higher mean haematocrit value than those in the satiation group, although this difference was not quite significant ($p = 0.056$) (Table 2). Fish that had lost weight (i.e., SGR < 0, $n = 3$) were not included in this data set.

Fish from both tanks were divided into three groups of equal size ($n = 8$) according to SGR (fast, medium, and slow growers), and the mean percentage of each meal obtained by the members of each of these groups was calculated. The feeding patterns of the fish in these groups for each of the daily meals are shown in Fig. 4. In the half-satiation group, fish that showed the fastest growth rates were also found to be those that consumed the largest portion of each of the three daily meals. In addition, the slowest growing fish were those that received the smallest portion of the meals. There was negligible variation in this relationship over the three daily meals (Fig. 4A). However, among satiation-fed fish, those who grew the fastest were not necessarily the same fish that obtained the largest portion of each meal, and there appeared to be much more variability in the group feeding patterns at different times (Fig. 4B).

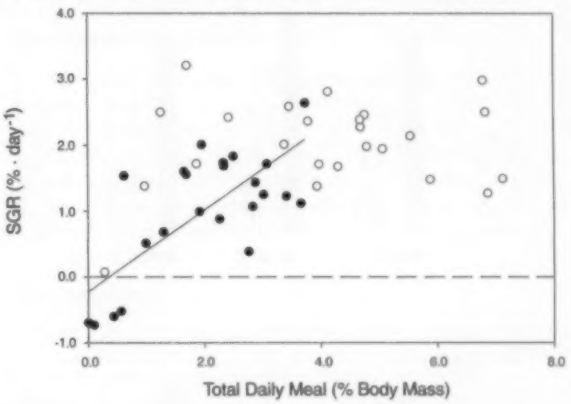

Fig. 3. Relationship between specific growth rate (SGR) and total daily meal among fish fed on satiation (open circles) and half-satiation (solid circles) rations. A significant positive regression was found between growth rate and meal size in the half-satiation diet group ($r^2 = 0.5413$, $p < 0.01$, $n = 24$), but not among fish fed to satiation. The regression line $y = 0.62x - 0.22$ represents the relationship found in the half-satiation group only.

Discussion

Swimming performance and growth rate

Individual rainbow trout raised together under food restriction show a significant negative relationship between SGR and relative U_{crit} ($r^2 = 0.2004$) and a significant positive relationship between SGR and stamina ($r = 0.3848$). These relationships occur at the level of individual variation and are not

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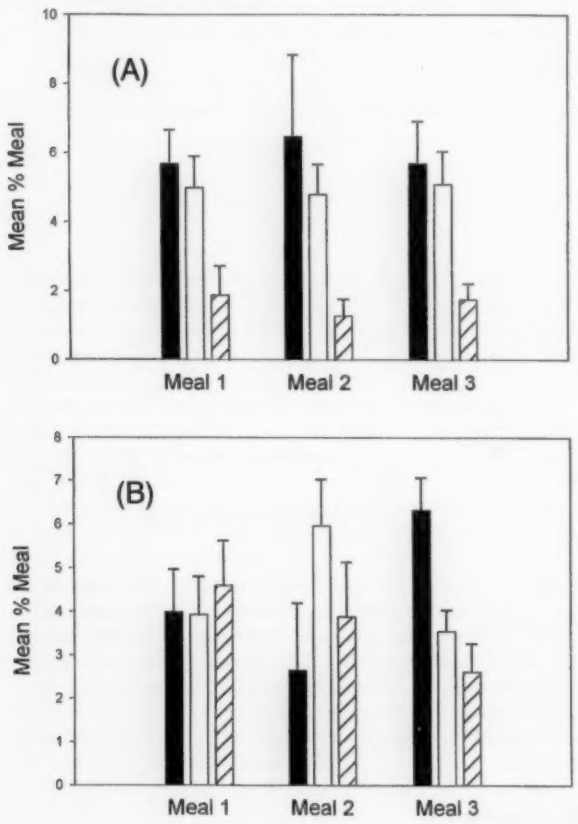
Table 2. Mean values ± SE (n) of mass, specific growth rate (SGR), coefficient of variation (CV), fin score, plasma cortisol levels, glycogen content, and haematocrit in the satiation and half-satiation diet groups of series 3.

Variable	Half-satiation	Satiation	p
Initial mass (g)	5.34±0.11 (25)	5.55±0.18 (25)	>0.32
Final mass (g)	7.85±0.46 (24)	11.05±0.54 (24)	<0.0001*
Final SGR (%·day^{-1})	1.04±0.19 (24)	2.03±0.14 (24)	<0.0001*
CV (%)	62.51±9.85 (24)	65.90±9.21 (24)	>0.80
Fin score	19.38±0.38 (24)	20.71±0.25 (24)	<0.006*
Plasma cortisol (μg·dL^{-1})	15.88±0.80 (22)	14.69±0.95 (22)	>0.34
Glycogen content (μmol glycosyl U·g wet weight^{-1})	10.84±1.37 (23)	10.24±1.39 (23)	>0.76
Haematocrit (% vol.)	41.48±0.61 (23)	39.55±0.78 (22)	>0.055

Note: CV is a measure of the variability in the food intake of individual fish from meal to meal. Differences in sample size are due to mortality and (or) problems with chemical analysis (such as an insufficient plasma sample in very small fish).

*Significant difference ($p < 0.05$).

Fig. 4. Mean percentage of each meal acquired by fish in fast- (solid bars), medium- (open bars), and slow-growing (hatched bars) groups (eight fish per group) for each of the three daily meals among fish fed (A) a half-satiation diet and (B) a satiation diet. Error bars represent standard error.



apparent when SGR is increased by feeding a greater ration or in the comparison of group means. They are seen most clearly under the intensified competition of a restricted ration.

It is important to consider whether these relationships could be spurious because of the observed intercorrelations between body size and growth rate and the well-known effects of size itself on swimming performance. In general, a *reduction* in relative U_{crit} is associated with greater size (e.g., Brett 1964; Hawkins and Quinn 1996) whereas an *increase* in fatigue time in fixed-velocity trials is associated with greater size (e.g., Brett 1967; Beamish 1978; McDonald et al. 1998). However, since there was no significant relationship between fatigue time and mass or other indices of body size in the present data, this was clearly not a complication in the positive SGR versus stamina relationship found among fish fed to half-satiation.

The situation is more complex for the negative relationship between SGR and relative U_{crit} present in the trout on the half-satiation diet because relative U_{crit} was also negatively correlated with body mass, fork length, and maximum cross-sectional area. However, forward stepwise linear regression analysis showed SGR to provide the most powerful explanatory model, and no significant relationships were found between the regression residuals (of U_{crit} versus SGR) and these other factors. More importantly, there was a significant regression between the residuals of the relationship between mass versus SGR and those of mass versus relative U_{crit}, indicating a nonspurious relationship between SGR and relative U_{crit}. In addition, absolute U_{crit} showed a significant positive relationship with body size, but not with SGR. These findings suggest that there is an association between relative U_{crit} and SGR independent of body size. This conclusion is supported by a recent study using transgenic coho salmon (*Oncorhynchus kisutch*) that showed an inordinate increase in growth rate and an associated reduction in aerobic swimming performance that could not be explained by differences in body size (Farrell et al. 1997). Kolok and Oris (1995) reported a negative correlation between SGR and *absolute* U_{crit} in fathead minnow (compared with the relationship between SGR and *relative* U_{crit} reported in the present study). These authors did not evaluate relative swimming performance, and the use of different swimming protocols is believed to be responsible for any discrepancies in the relationships between SGR and absolute U_{crit} reported in the two studies (T.R. Gregory and C.M. Wood, unpublished results).

There are several possible, and in no way mutually exclusive, explanations for this antagonistic relationship between SGR and relative U_{crit}. These include changes in morphology (Taylor and McPhail 1985) associated with rapid growth, excessive energetic inputs required for white muscle synthesis in fast-growing fish (Loughna and Goldspink 1984), the

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metabolic cost of specific dynamic action (SDA) associated with feeding (e.g., Jobling and Davis 1980; Alsop and Wood 1997), the effects of lower haematocrit on swimming performance (e.g., Gallaugher et al. 1995), and insufficient aerobic enzyme activity in fast-growing fish (Somero and Childress 1980; Pelletier et al. 1993*a*).

The explanation for the positive relationship between SGR and stamina may be more straightforward, considering that the stamina test provides a measure of anaerobic capacity (McDonald et al. 1998). As a general paradigm, the activities of glycolytic enzymes tend to be greater in larger fish (Somero and Childress 1980), although there was no relationship between stamina and body size in the present study (only between stamina and SGR). In accord with this finding, Pelletier et al. (1993*b*) have shown that the activities of several key glycolytic enzymes are positively correlated with growth rate but not with body size in Atlantic cod (*Gadus morhua*). As such, faster growing fish may have more efficient anaerobic metabolisms than slower growing individuals. The absence of a significant relationship between growth rate and glycogen levels in the present study suggests that the efficiency with which an individual carries out glycolysis, and not the concentration of anaerobic substrates, may be the more important determinant of anaerobic swimming performance. It is also possible that the positive relationship between SGR and stamina in the half-satiation diet is directly "causative." The capacity for rapid, short-term activity measured by the fixed-velocity fatigue test may be related to the abilities needed to acquire food more effectively, and therefore grow more rapidly, in a highly competitive, limited-ration situation (Fig. 3).

Feeding and growth rate

In both series 2 and 3, fish that were fed a satiation diet showed significantly higher mean growth rates than fish fed a half-satiation diet. Obviously, the availability of food is an important determinant of growth rate, since the energy required for growth is acquired in the diet. At high levels of feeding, however, growth efficiency declines with increases in ration, due primarily to an increase in SDA (Wurtsbaugh and Davis 1977). There may be a phenomenon of "diminishing returns" associated with high levels of feeding, whereby fish that consume large amounts of food do not grow as efficiently as fish fed a smaller ration.

The significant difference in fin score between the two groups suggests that fish fed a half-satiation diet experienced more aggression than fish fed to satiation (Abbott and Dill 1989), probably as a result of more intense competition for food. However, mean plasma cortisol levels were not significantly different and SGR was not directly correlated with fin score in either group.

In addition to ration, feeding schedule may affect the feeding patterns of fish. Other researchers have reported significant differences in CV among fish fed different rations, with low rations resulting in more variable individual feeding patterns (McCarthy et al. 1992). However, no significant differences in CV were found between the two diet groups in this study. This difference appears to be due to the use of three daily meals rather than a single large daily meal (T.R. Gregory and C.M. Wood, unpublished results). As is evident from Fig. 4, the feeding patterns of the diet groups were quite different. The fastest growing fish were not always those who acquired the most food in each of the three daily meals in the satiation group whereas in the half-satiation group, the fastest growing fish received the largest portion of the meal in all three cases. This difference probably results from the more strict enforcement of a feeding hierarchy in the half-satiation group, with the same individuals acquiring most of the food each time.

Limits to fish growth

In addition to the well-known advantages inherent in rapid growth, such as earlier reproduction (Metcalfe et al. 1989), improved access to preferred sites (Fausch 1984), and more flexible food sources (Webb et al. 1984), the present results indicate another potential benefit: greater stamina. Nevertheless, the growth of fish is ultimately limited by several important aspects of physiology and ecology, including increased predation risk associated with the heightened appetite and foraging behaviour stimulated by high levels of growth hormone, increased occurrence of developmental errors associated with rapid growth, and the investment in growth occurring at the expense of somatic maintenance and repair (Johnsson and Björnsson 1994). Rapid growth may also be limited by the energetic cost associated with high levels of feeding, digestion, and food conversion. The results of the present study offer yet another explanation: that rapid growth occurs to the detriment of another key element of fitness, aerobic swimming performance.

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Biochemical correlates of growth and condition in juvenile Atlantic cod (*Gadus morhua*) from Newfoundland

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Abstract: The aim of this study was to examine how the biochemical composition of tissues varied with growth rate and condition in juvenile Atlantic cod (*Gadus morhua*) caught in the wild and kept in captivity. The hepatosomatic index, brain water content, and muscle sarcoplasmic protein content as well as the activities of phosphofructokinase, lactate dehydrogenase, nucleoside diphosphate kinase, and citrate synthase in the muscle, nucleoside diphosphate kinase and citrate synthase in the intestine, and cytochrome *c* oxidase and citrate synthase in the brain increased with growth rate or condition factor. Conversely, liver and muscle water contents were lower in fish with a higher growth rate. A multiple regression model that included the hepatosomatic index, water content of muscle and brain, and citrate synthase activity in the intestine explained 79.7% of the variability of growth in mass under our conditions. A similar model, using liver water content instead of muscle water content, explained 82.5% of the variability of growth in length. These easy to measure variables may be used in fisheries management to estimate the growth rate of fish in the wild.

Résumé : Cette étude a pour but d'examiner comment la composition biochimique des tissus varie en fonction du taux de croissance et de la condition de morues franches (*Gadus morhua*) capturées en mer et gardées en captivité. L'indice hépatosomatique, la teneur en eau du cerveau et la teneur en protéines sarcoplasmiques des muscles de même que les activités de la phosphofructokinase, de la lactate déshydrogénase, de la nucléoside diphosphate kinase et de la citrate synthase dans le tissu musculaire, de la nucléoside diphosphate kinase et de la citrate synthase dans l'intestin ainsi que de la cytochrome *c* oxydase et de la citrate synthase dans le cerveau s'accroissaient avec le taux de croissance ou le coefficient de condition. Inversement, les teneurs en eau du foie et du tissu musculaire étaient plus faibles chez les poissons ayant un taux de croissance élevé. Un modèle de régression multiple prenant en considération l'indice hépatosomatique, la teneur en eau du tissu musculaire et du cerveau et l'activité de la citrate synthase dans l'intestin rendait compte de 79,7% de la variabilité de la croissance en masse dans nos conditions expérimentales. Un modèle similaire, utilisant la teneur en eau du foie au lieu de la teneur en eau du tissu musculaire, rendait compte de 82,5% de la variabilité de la croissance en longueur. Ces variables faciles à mesurer peuvent être utilisées dans la gestion des pêches pour estimer le taux de croissance des poissons dans leur milieu naturel.

[Traduit par la Rédaction]

Introduction

In the late 1980s and early 1990s, many Atlantic cod (*Gadus morhua*) stocks in the Northwest Atlantic declined markedly. Several explanations were put forward to explain these declines, including recruitment overfishing (Hutchings and Myers 1994; Myers and Cadigan 1995) and predation by seal populations (Hammill and Stenson 1997). The remaining fish are smaller, exhibit slower growth rates, and reach much lower levels of condition than those in the past (Chouinard and Fréchet 1994; Lambert and Dutil 1997*a*). Growth rate is a key variable in the recovery of cod stocks, but it is difficult to quantify in the wild.

Variations in growth rate bring about changes in the biochemical composition of fish tissues (Houlihan et al. 1993; Guderley et al. 1996). Many metabolic variables have also been reported to follow the growth in mass of fish kept in the laboratory. These include, for cod, indicators of aerobic and glycolytic capacities of muscle, liver, and intestine (Houlihan et al. 1993; Pelletier et al. 1993*a*, 1993*b*, 1995).

Growth involves two sets of processes that vary in relative importance depending on the developmental stage of the animal. Growth in length appears to dominate in young fish, while growth in mass becomes relatively more important as fish reach adulthood (Ricker 1979). Growth in length involves elongation of bone, muscle, and other tissues and necessarily involves the synthesis of structural and functional molecules and cell differentiation. While growth in mass may result from growth in length and its associated increase in mass, it also reflects the accumulation of energy reserves in the form of lipids in the liver (Black and Love 1986; Jobling 1988; Lambert and Dutil 1997*b*) and protein in the muscle (Black and Love 1986; Guderley et al. 1996; Lambert and Dutil 1997*b*). Growth in length can be considered as more permanent than

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growth in mass, since food deprivation typically leads to loss of body mass, while reduction of length has not, to our knowledge, been reported. As longer fish have the potential to gain more mass during favourable conditions, growth in length may enhance the potential for future productivity. In this study, we compare the relationships of various metabolic capacities in cod tissues with growth in length and with growth in mass. We hypothesize that the activity of enzymes more directly involved in biosynthesis (such as nucleoside diphosphate kinase (NDPK)) will show a stronger dependence on growth in length. Conversely, higher glycolytic capacities (estimated here with the activities of phosphofructokinase (PFK) and lactate dehydrogenase (LDH)) appear to result from growth in mass (Pelletier et al. 1993*a*) and we predict that they will show a stronger dependence on growth in mass.

For this study, juvenile cod were fed different rations to achieve a range of growth rates over the experimental period. Growth rate in mass and in length as well as condition factor and hepatosomatic index were calculated for each fish and a series of biochemical measurements made on their liver, intestine, white axial muscle, and brain to determine tissue composition (water and protein contents) and metabolic capacities. We included brain in our study, since this tissue, contrary to muscle and liver, is not involved in accumulating energy reserves and hence has a high potential to show growth-related variations in composition that are independent of the energetic status of the fish. In a composite model aiming to predict growth, variations in brain composition could account for a portion of the interindividual variability in growth that is not accounted for by information from other tissues.

No study has assessed the potential for a combination of metabolic capacities and tissue biochemical composition to predict the instantaneous growth rate of cod. These models could allow the estimation of recent growth rate of individual cod in the wild from discrete samples as opposed to repetitive samplings such as are used in mark–recapture studies. We have built models with parameters that vary with growth in length or in mass to examine their potential as growth rate estimators.

Materials and methods

Fish

Juvenile Atlantic cod ($n = 62$) were caught off the eastern coast of Newfoundland at the end of August 1994 and shipped by aircraft to Dalhousie University, Halifax, N.S. Four weeks later, they were shipped by aircraft to Institut Maurice-Lamontagne. They were allowed to recover for 8 weeks at temperatures increasing gradually from 0 to 4°C. During this period, animals were fed capelin (*Mallotus villosus*) three times a week to satiety. At the end of this period, 13 fish were killed, measured, and their organ, carcass, and total masses recorded. These fish were used to set up the enzyme and protein assays.

Growth rate experiments and tissue sampling

At the beginning of December 1994, the remaining 49 fish (all apparently healthy) were anesthetized for about 5 min in 60 L of water containing 5 mg metomidate hydrochloride·L^{-1}. Each fish was then measured, weighed, and labelled with visual implant tags (Northwest Marine Technology, Wash.) inserted in the dorsal fin. These fish were placed in four 1.5-m^3 circulating tanks at 4°C as follows: 10 fish in tank 1, 15 fish in tank 2, 14 fish in tank 3, and 10 fish in tank 4. Cod in tanks 2 and 3 were fed capelin three times a week to satiety; those in tank 1 were fed once weekly to satiety and those in tank 4 received half the feed ingested by fish in tank 1. Fish were kept under these conditions for 84 days, during which time, no mortality was observed.

At the end of the growth experiments, the fish were killed by a blow to the head, measured, weighed, and the mass of their carcasses, livers, and cleaned intestines recorded. A 0.5-cm^3 sample of white muscle was excised from below the dorsal fin, on the left side, 0.5 cm under the skin. These samples as well as a sample of liver and the whole brain and cleaned intestine were frozen in liquid nitrogen and transported to the Département de Biologie, Université Laval, on dry ice where they were stored at –80°C for further analyses.

Water content determinations

White muscle, liver, brain, and intestine samples were dried to a constant mass at 60°C for 48 h and water content expressed as milligrams of water per gram of tissue.

Protein assays

White muscle proteins were separated into sarcoplasmic (soluble) and insoluble structural (including myofibrillar and extracellular) protein fractions following the method of Somero and Childress (1990), with the modification that 1 mL of 1/10 homogenates in low-salt phosphate buffer (50 mM, pH 7.0, +0.1% Triton X-100) was centrifuged at 13 000 × *g* in Eppendorf tubes using an IEC Micromax microcentrifuge. Total, sarcoplasmic, and structural protein samples from white muscle as well as total protein of liver, brain, and intestine homogenates in 100 mM phosphate buffer were assayed using the bicinchoninic acid method of Smith et al. (1985), using bovine serum albumin as standard.

Enzyme assays

Tissue samples were homogenized as described in Pelletier et al. (1993*a*). All enzyme assays were performed at 10°C (which is close to the thermal optimum for growth for the species) using a UV/Vis spectrophotometer (Beckman DU640) equipped with a thermostatted cell holder and circulating refrigerated water bath. Cytochrome *c* oxidase (CCO) activity was measured at 550 nm to follow the oxidation of reduced cytochrome *c*. The millimolar extinction coefficient of cytochrome *c* was 19.1. Citrate synthase (CS) activity was monitored at 412 nm to detect the transfer of sulphydryl groups from coenzyme A to 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). The nanomolar extinction coefficient of DTNB was 13.6. Activities of PFK, NDPK, and LDH were monitored at 340 nm to follow the oxidation of NADH. The millimolar extinction coefficient of NADH was 6.22. Assays were conducted in duplicate and results are expressed in international units (micromoles of substrate converted to product per minute). Assay conditions for PFK (EC 2.7.1.11), CS (EC 4.3.1.7), CCO (EC 1.9.3.1), and LDH (EC 1.1.1.27) were as described in Pelletier et al. (1994), with the following modifications. For PFK, 1 mM ATP and AMP were used and for LDH, 0.5 mM pyruvate was used. NDPK (EC 2.7.4.6) assay conditions were as follows: imidazole–HCl, 50 mM; $MgCl_2 \cdot 6H_2O$, 20 mM; KCl, 70 mM; NADH, 0.24 mM; ATP, 2 mM (muscle and brain) or 1 mM (intestine); phosphoenolpyruvate, 1.1 mM; excess LDH and pyruvate kinase; thymidine diphosphate, 0.7 mM (muscle and brain) or 1.4 mM (intestine); pH 7.2. Thymidine diphosphate was omitted for control.

Multiple regression equation building

We constructed multiple regression models to determine which combination of variables best explained the variability of growth rate in length or in mass. Models were built using JMP 2.0 software (SAS Institute Inc., Cary, N.C.) as follows. First, all variables that individually showed a significant ($p < 0.05$) linear relationship with growth rate were used in a multiple regression model. In the next step, every combination of these 10 variables was examined in multiple regression models including two to six variables. The model with the highest correlation coefficient but the lowest number of variables was selected. In the last step, the resulting model was modified by entering

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Table 1. Mean values (range in parentheses) of cod condition, tissue composition, and enzyme activities measured in swimming muscle, liver, intestine, and brain of 49 experimental cod.

Variable	Cod	Muscle	Liver	Intestine	Brain
General					
Initial length (mm)	288 (230–360)				
Final length (mm)	326 (250–410)				
Initial mass (g)	232 (79–500)				
Final mass (g)	331 (117–753)				
Initial condition factor	0.93 (0.65–1.12)				
Final condition factor	0.91 (0.67–1.13)				
Growth rate (% mass gain·day^{-1})	0.51 (–0.15–1.33)				
Growth rate (% length gain·day^{-1})	0.15 (0.03–0.28)				
Final HSI	4.56 (1.74–10.40)				
Tissue composition (mg·g wet mass^{-1})					
Total water		799 (785–821)	366 (262–712)	811 (794–837)	784 (760–812)
Total protein		140.1 (117.1–167.9)	63.2 (29.9–105.4)	83.3 (58.2–116.6)	61.0 (32.9–87.8)
Structural protein		94.9 (75.3–116.4)	—	—	—
Sarcoplasmic protein		45.2 (31.9–55.5)	—	—	—
Enzyme activity (IU·g wet mass^{-1})					
PFK		5.62 (0.16–13.92)	—	—	2.51 (0.69–3.77)
PK		67.1 (26.6–144.1)	—	—	29.3 (18.8–38.4)
LDH		196.7 (61.4–299.2)	226.6 (133.6–310.9)	—	40.2 (30.0–53.0)
CS		1.78 (0.66–3.63)	0.86 (0.28–1.81)	3.52 (2.43–4.95)	7.83 (6.07–10.13)
CCO		1.49 (0.39–3.20)	4.90 (1.32–13.29)	9.00 (2.90–14.00)	8.41 (2.72–16.64)
NDPK		127.0 (63.0–174.8)	—	72.8 (50.1–110.5)	—

Note: If parameters are not indicated, they were not determined for the tissue in question.

individually other variables that had shown a significant correlation with growth rate to ensure that no other variable would contribute to the model. When entering a new variable, the significance level of its F ratio was examined. This significance level indicated the contribution of that variable to the model. For example, when entering a variable that covaried with another one already in the model, the probability level of the F ratio of that new variable was typically above 0.20, indicating that it contributed little to the model. Before the last variable was entered, every variable already in the model had a significance level of its F ratio below 0.02. For the last variable entered, a significance level of its F ratio around 0.06 was accepted, since it increased the proportion of the variability of growth rate explained by the model by at least 1.6%.

Calculations and statistics

Growth rates were calculated as the relative increase in body mass and body length over the 84-day period of the experiment and are expressed as daily percent increase (Ricker 1979). Fulton's condition factor was calculated as the mass of the fish at the end of the growth experiments divided by the cubed length. The hepatosomatic index (HSI) was calculated as the mass of liver divided by the total mass of these juvenile fish (gonad mass was negligible). Linear regressions and their levels of significance were established using JMP 2.0 software (SAS Institute Inc.). No allometric corrections were needed, since those corrections that could be applied to our data by using equations from Pelletier et al. (1993*b*) and Martinez (1996) did not alter our conclusions (data not shown).

Results

A range of growth rates was achieved by juvenile Atlantic cod over the 84-day experimental period, both due to the various levels of food rationing among the four tanks and presumably because of competition and variation in individual performance. Growth in mass varied from slightly negative to 1.33% mass gain·day^{-1} (Table 1). In contrast, growth in length was positive in all cases, although rates varied considerably between individuals. As expected, there was a strong relationship between growth in mass and growth in length ($R^2 = 0.889$, $p < 0.0001$). No significant relationship was found between initial condition factor and the subsequent rate of growth. At the end of the growth experiment, however, a strong relationship existed between growth rate in mass or in length and condition factor ($R^2 = 0.511$ and 0.494, respectively). Nevertheless, average condition factor of juvenile cod did not change during the growth experiment.

Tissue and body composition and metabolic capacities versus growth rate and condition factor

Tissue water and protein contents and enzyme activities determined for white muscle, liver, intestine, and brain varied among fish (Table 1), and many of these parameters showed significant relationships with growth rate and condition factor (Tables 2 and 3). Most variables examined showed a slightly stronger relationship with growth rate in length than in mass.

Among the variables examined, the HSI was the most strongly correlated with growth rate in mass or in length (Table 2). HSI alone explained 69 and 72% of the variability of growth rate in mass or in length, respectively. HSI was also strongly correlated with condition factor.

There were significant relationships between muscle, liver, and brain water contents and growth in both mass and length as well as with condition factor (Table 2). Water content of muscle and liver decreased with increasing growth rate, while the opposite was observed for brain. Tissue water content explained a significant proportion of the variability in growth rate

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Table 2. Linear regressions between tissue water (mg·g tissue^{-1}) and protein (total or sarcoplasmic for muscle) (mg·g wet tissue^{-1}) and growth in mass, growth in length, or condition factor (y) in juvenile Atlantic cod.

Variable	Growth in mass (% gain·day^{-1})	Growth in length (% gain·day^{-1})	Condition factor
HSI	$y = 2.54 + 3.72x$ ($R^2 = 0.686$, $p < 0001$)	$y = 0.9 + 23.1x$ ($R^2 = 0.724$, $p < 0001$)	$y = -7.2 + 12.9x$ ($R^2 = 0.638$, $p < 0001$)
Water			
Muscle	$y = 804.8 - 10.8x$ ($R^2 = 0.270$, $p < 0.0001$)	$y = 811.6 - 79.3x$ ($R^2 = 0.403$, $p < 0.0001$)	$y = 838.6 - 43.3x$ ($R^2 = 0.360$, $p < 0.0001$)
Liver	$y = 455.0 - 173.7x$ ($R^2 = 0.415$, $p < 0.0001$)	$y = 554.7 - 1221.6x$ ($R^2 = 0.565$, $p < 0.0001$)	$y = 961.0 - 655.8x$ ($R^2 = 0.488$, $p < 0.0001$)
Brain	$y = 774.7 + 17.3x$ ($R^2 = 0.243$, $p < 0.0005$)	$y = 767.4 + 104.3x$ ($R^2 = 0.245$, $p < 0.0005$)	$y = 750.1 + 36.8x$ ($R^2 = 0.091$, $p < 0.05$)
Protein			
Muscle (total)	$y = 134.2 + 11.5x$ ($R^2 = 0.110$, $p < 0.05$)	$y = 127.8 + 79.3x$ ($R^2 = 0.144$, $p < 0.01$)	$y = 103.0 + 40.9x$ ($R^2 = 0.115$, $p < 0.05$)
Muscle (sarcoplasmic)	$y = 41.3 + 7.7x$ ($R^2 = 0.272$, $p < 0.0001$)	$y = 37.4 + 50.1x$ ($R^2 = 0.320$, $p < 0.0001$)	$y = 17.4 + 30.7x$ ($R^2 = 0.360$, $p < 0.0001$)
Liver	$y = 78.6 - 30.0x$ ($R^2 = 0.317$, $p < 0.0001$)	$y = 94.7 - 204.0x$ ($R^2 = 0.404$, $p < 0.0001$)	$y = 162.3 - 109.2x$ ($R^2 = 0.348$, $p < 0.0001$)

Table 3. Linear regressions between enzyme activities (units·g wet tissue^{-1}) and growth in mass, growth in length, or condition factor (y) in tissues of juvenile Atlantic cod.

Variable	Growth in mass (% gain·day^{-1})	Growth in length (% gain·day^{-1})	Condition factor
Muscle			
PFK	ns	$y = 3.20 + 15.65x$ ($R^2 = 0.080$, $p < 0.05$)	$y = -5.06 + 11.73x$ ($R^2 = 0.136$, $p < 0.01$)
LDH	$y = 146.6 + 97.9x$ ($R^2 = 0.355$, $p < 0.0001$)	$y = 100.4 + 624.1x$ ($R^2 = 0.397$, $p < 0.0001$)	$y = -188.5 + 424.6x$ ($R^2 = 0.551$, $p < 0.0001$)
NDPK	$y = 111.8 + 29.7x$ ($R^2 = 0.160$, $p < 0.005$)	$y = 92.7 + 222.4x$ ($R^2 = 0.248$, $p < 0.0005$)	$y = 56.6 + 77.6x$ ($R^2 = 0.091$, $p < 0.05$)
CS	$y = 1.41 + 0.71x$ ($R^2 = 0.209$, $p < 0.001$)	$y = 1.17 + 3.94x$ ($R^2 = 0.180$, $p < 0.005$)	$y = -0.19 + 2.16x$ ($R^2 = 0.162$, $p < 0.005$)
Intestine			
NDPK	$y = 64.9 + 15.5x$ ($R^2 = 0.160$, $p < 0.005$)	$y = 59.5 + 86.1x$ ($R^2 = 0.135$, $p < 0.01$)	$y = 33.4 + 43.5x$ ($R^2 = 0.104$, $p < 0.05$)
CS	$y = 3.09 + 0.09x$ ($R^2 = 0.262$, $p < 0.0005$)	$y = 2.77 + 4.88x$ ($R^2 = 0.235$, $p < 0.0005$)	$y = 1.89 + 1.80x$ ($R^2 = 0.095$, $p < 0.05$)
Brain			
CCO	ns	$y = 5.4 + 19.6x$ ($R^2 = 0.109$, $p < 0.05$)	ns
CS	ns	$y = 7.13 + 4.53x$ ($R^2 = 0.086$, $p < 0.05$)	ns

Note: Only relationships with $p < 0.05$ are shown; ns, not significant.

or condition. However, water content of the brain was only weakly correlated with condition factor.

Total muscle proteins can be divided into two categories: sarcoplasmic (soluble) proteins and structural (myofibrillar and extracellular). In muscle, total protein content is the sum of sarcoplasmic and structural proteins. Sarcoplasmic protein content of muscle increased significantly with growth in length and in mass and with condition (Table 2). Conversely, structural protein content was independent of growth rate or condition (not shown). For this reason, the relationship of total protein content with growth rate or condition was weaker than that of sarcoplasmic protein. Protein content of liver decreased with increasing growth rate in a similar pattern as that observed for water content, hence likely reflecting a parallel increase in lipid content. Intestine and brain protein contents did not vary with growth rate or condition.

In muscle, the activities of four enzymes from different metabolic pathways increased with growth rate. The activities of a glycolytic enzyme, LDH, a mitochondrial enzyme, CS, and a biosynthetic enzyme, NDPK, all increased with growth in mass or in length (Table 3). The activity of PFK only increased with growth rate in length. Both glycolytic enzymes, PFK and LDH, showed a stronger relationship with condition factor than with growth in mass or in length, while NDPK and

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CS were more strongly related to growth rates. Since PFK and LDH are sarcoplasmic proteins, they are likely part of the explanation for the increase in sarcoplasmic protein content with growth (Table 2). Indeed, the activities of PFK, LDH, and CS increased significantly with muscle sarcoplasmic protein content (R^2 = 0.113, 0.487, and 0.200, respectively). Moreover, the ratio of LDH to muscle sarcoplasmic protein increased significantly with increases in growth rates in mass or in length (R^2 = 0.274 and 0.301, respectively). The ratio of CS activity to muscle sarcoplasmic protein only increased with increases in growth rate in mass (R^2 = 0.098).

As for white muscle, intestine NDPK and CS activities increased with growth in mass and in length (Table 3). The relationships of NDPK and CS activities with the condition factor were considerably weaker than those with growth. Another indicator of aerobic capacity, CCO, apparently decreased with growth rate, but this observation was likely due to an artifact. Indeed, unusually low activities of CCO (below 5 units·g wet mass^{-1}) were measured in the intestine of five fish with high growth rates, and by excluding these measurements, no correlation could be established between CCO activity in the intestine and growth rate or condition factor.

No significant relationships of enzyme activities with growth in mass or with condition factor could be established in the brain. However, both indicators of aerobic capacity, CCO and CS, increased significantly with growth in length (Table 3).

The decrease in liver water and protein contents with growth rate and condition factor suggests an important increase in lipid content. Both indicators of aerobic capacity, CCO and CS, showed a significant decrease in activity per gram of tissue with growth or condition factor ($p < 0.0001$, not shown). Hepatic LDH activity also decreased with growth in mass ($p < 0.01$) but not with growth in length. In a further analysis of the data, enzyme activities were expressed as units per gram of lipid-free liver, using an estimation of the mass of lipid per gram of liver by subtracting the water and protein contents measured and assuming that ashes were a negligible portion of liver wet mass. CCO and CS activities in lipid-free liver remained lower in fish with a high growth rate or condition factor. However, LDH activity expressed per gram of lipid-free liver showed a significant increase in fish with a higher growth rate in mass or in length as well as in fish with a higher condition factor (R^2 = 0.083, 0.218, and 0.169, respectively).

Multiple regression models explaining the variability of growth rate

The most important correlate of growth was HSI (Table 2), which alone explained 69 or 72% of the variability of growth rate in mass or in length, respectively (Tables 2 and 4). Water and protein contents of tissues were also good indicators of growth rate, as were some enzyme activities (Tables 2 and 3). The optimal multivariate models explaining the variability of growth rate included different variates from several tissues. HSI, water contents of muscle and brain, and CS activity in the intestine together explained 79.7% of the variability of growth rate in mass (Table 4). This was about 10% more than HSI alone. Adding more variables did not increase the coefficients of determination to a level that could justify their inclusion in the model. Not including muscle or brain water content decreased the coefficient of determination to 0.779 or 0.739, respectively. A model that used HSI and the water contents of muscle and brain but not CS activity in the intestine gave a coefficient of determination of 0.753. Finally, excluding HSI but keeping the water contents of muscle and brain and the CS activity of the intestine gave a model with a coefficient of determination of only 0.527. Other variates measured in the liver could be included in a model to partly compensate for the exclusion of HSI. A model including the water contents of muscle, liver, and brain and CS activity in the intestine could explain 69.1% of the variability of growth rate in mass.

Table 4. Multiple regression models utilizing the variables that best explain the variability of growth rate in mass and in length in juvenile cod.

Variable	y = % mass gain·day^{-1}	y = % length gain·day^{-1}
R^2	0.797	0.825
Intercept	–14.34820	–0.87526
HSI	0.17545 (68.6%)	0.01941 (72.4%)
mg water·g wet muscle^{-1}	0.00990 (1.8%)	—
mg water·g wet liver^{-1}	—	–0.00015 (2.9%)
mg water·g wet brain^{-1}	0.00723 (5.9%)	0.00121 (5.6%)
Units CS·g wet intestine^{-1}	0.14250 (3.4%)	0.01404 (1.6%)

Note: Numbers in parentheses represent the percentage of residual variability of growth rate explained by this variable when entered in the model. Refer to the Materials and methods section for details on model building.

Our models were slightly better at predicting growth in length than growth in mass. The best combination of variables for a model explaining the variability of growth in length was similar to that for growth in mass, except that muscle water content was replaced by liver water content (Table 4). This model could explain 82.5% of the variability of growth in length. Including muscle water content, as in the model for predicting growth in mass, did not alter the coefficient of variation (R^2 = 0.827). However, removing the water content of liver or brain decreased the coefficient of determination to 0.800 or 0.767, respectively. A model excluding intestinal CS activity yielded a coefficient of determination of 0.809. Finally, a model using water contents of liver and brain and CS activity in the intestine but not HSI yielded a coefficient of determination of 0.718.

We examined how well the variables selected as best available predictors of growth rate could explain the variability of condition factor. A model made with the variables that explained 79.7% of the variability of growth in mass (Table 4) explained only 64.3% of the variability of condition factor. The variables that explained 82.5% of the variability of growth in length could only explain 66.8% of the variability of condition factor.

Discussion

Biochemical changes in growing juvenile cod

Muscle protein increased and water content decreased with increases in growth rate or condition, suggesting that protein replaced some of the water in fish with higher growth rates. For growth rates from 0 to 1.5% mass gain·day^{-1}, muscle water content decreased from 80.5 to 79%, while protein content

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increased from 13.5 to 15%. Muscle structural protein content remained remarkably constant despite variations in condition and growth rate; the sarcoplasmic protein fraction increased with growth rate. A similar response of these muscle protein fractions to changes in growth and condition has been found for cod from the Gulf of St. Lawrence (Guderley et al. 1996). Muscle proteins play several roles in fish. They provide the machinery and the structure required for locomotion as well as representing an energetic reserve. Starvation leads to considerably greater increases in fish muscle water content than in that of terrestrial vertebrates, with water contents increasing from 79 to as much as 90% in large cod (Love 1980). Our results suggest that structural proteins are retained even when their energetic content could be useful. Their retention may allow the fish to benefit from increases in food availability, after having replaced the bulk of its sarcoplasmic proteins and glycogen by water. Similar patterns are found in other species. During the spawning migration of sockeye salmon (*Oncorhynchus nerka*), soluble proteins decline more than insoluble proteins (Mommsen et al. 1980). Androgen stimulation of chum salmon (*Oncorhynchus keta*) leads primarily to mobilization of sarcoplasmic proteins (Ando et al. 1986). Changes in the feeding frequency of cod modify sarcoplasmic proteins more than structural proteins (von der Decken and Lied 1992).

The activities of CS and LDH have been shown to increase with growth rate (Pelletier et al. 1993*a*, 1993*b*, 1994). Our results also show that these catabolic enzymes increased faster than the total pool of muscle sarcoplasmic proteins, suggesting a specific enhancement in the activities of some enzymes relative to sarcoplasmic proteins in fast-growing cod, possibly to assist in the growth process. Muscle NDPK, an enzyme involved in biosynthesis, also increased with growth rate, but its variation in activity did not follow that of sarcoplasmic protein content. Hence, enzymes of anabolic pathways may differ from those of catabolic pathways in their relationship with the growth process.

Lipid accumulation in liver is typical of growing cod (Black and Love 1986) and leads to high values of HSI. In this experiment, fish with a higher growth rate or condition factor showed a pronounced decrease in liver water and protein contents per gram of liver (Table 2), which was in all likelihood due to an increase in liver lipid content. The activities of all enzymes measured (LDH, CS, and CCO) expressed on a wet mass basis also decreased with increasing growth rate. However, an examination of LDH activity per unit mass of lipid-free liver suggested an increase in cellular activity of this enzyme in fish with higher growth rate or condition. This suggests that fish with a higher growth rate or condition factor increase their capacity for hepatic gluconeogenesis from blood lactate, which is consistent with the higher muscle LDH activity in fast-growing cod.

The intestine responded differently to growth than the other tissues examined. While intestinal water and protein contents remained independent of growth rate or condition factor, enzyme activities were influenced by both growth and condition (Table 3). Some fish, like largemouth bass (*Micropterus salmoides*) (Goolish and Adelman 1987), increase the relative mass of their intestine with higher growth rates. In other species like rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*) (Weatherley and Gill 1983) or cod (Pelletier et al. 1994), the digestive tract does not vary in relative mass or length with different feeding regimes. For these fish, variations in food availability and resulting variations in growth rate should be parallelled by changes in intestinal metabolic capacity. Pelletier et al. (1994) found that large cod growing at a faster rate increased the activity of CCO in their intestine and proposed that the aerobic capacity of the intestine may limit the rate of growth. Our study did not show a relationship between intestinal CCO activity and growth rate or condition. However, the activities reported by Pelletier et al. (1994) for CCO ranged from 2 to 8 units CCO·g wet mass^{-1} for fish with low to high growth rates, respectively. The activities measured in this study ranged from 6 to 14 units CCO·g wet mass^{-1} and are perhaps due to the smaller size of our fish. The majority of the fish examined in our experiment may have had sufficient intestinal CCO activity to cope with the increasing intestinal uptake associated with high growth rates. Hence, intestinal CCO activity would only be an indicator of recent growth for adult cod. The other mitochondrial enzyme (CS) and the biosynthetic enzyme (NDPK) measured in the intestine showed a marked increase in activity with growth rate (Table 3). The stronger relationship of CS and NDPK activities with growth than with condition factor (Table 3) supports the concept of symmorphosis (Taylor and Weibel 1981), which proposes that metabolic capacity is tightly linked to, and varies with, demand.

The tissue composition and metabolic capacities of brain also varied with growth rate, albeit not as much as for muscle or liver. The significance of the observed increase in water content in the brain of juvenile cod displaying higher growth rates (Table 2) is unknown. Since the protein concentration of the brain did not change with growth rate, it is possible that the lipid content of the brain decreased with increasing growth rate and that these lipids were replaced by water. This novel finding needs further investigation, particularly since our data show that condition factor has little influence on brain water content, making this parameter a potentially useful variable in the estimation of growth rate for cod.

Growth in length versus growth in mass and condition factor

An important limitation of studies on the impact of growth on metabolic capacities is that it is difficult to differentiate between enhancements of metabolic capacities that result from growth and those that are required for growth. Ideally, biochemical indicators of growth should belong to the latter category and these indicators should be proportional to growth rate and independent of recent growth history, or condition. Growth in mass likely influences metabolic capacities when growing fish restore their protein stores, including some soluble enzymes. Hence, enzymes for which the activities increase as a result of growth may not be directly involved in the growth process, although their increased activities may facilitate future growth. Our study shows that the activities of LDH and PFK in muscle are more related to the condition factor of the cod than to growth. It is possible that muscle LDH stores are depleted in periods of fasting and then restored when food is available and that the resulting higher muscle LDH activities favour further growth by enhancing swimming capacity.

Questions about the relative influence of fish condition and growth rate on metabolic capacities and tissue composition prompted us to examine the relationship of these parameters

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with growth in length. Growth in length partly involves different physiological processes than growth in mass, as the former implies the synthesis of structural molecules, while an important part of growth in mass may be due to the restoration and accumulation of energy reserves. The distinction between these types of growth may be subtle, but in physiological terms, they involve different processes. Seeking to identify metabolic parameters that reflect growth rate independently of the condition of the fish, we have compared their coefficients of correlation with growth in length and in mass and with condition factor. During the course of our growth experiment, some fish decreased in mass and condition, while others increased in mass and condition, but all of them grew in length, albeit at different rates. Our results suggest that the increase in the activity of an indicator of biosynthesis (NDPK) in muscle is directed towards growth, as this enzyme shows a stronger dependence on growth rate than on condition factor and is even independent of the increase of muscle sarcoplasmic protein, which occurs as a result of growth. The strong dependence of NDPK activity in the muscle on growth in length was expected, given the involvement of this enzyme in the biosynthesis required for this process. Another novel finding from this study is that the activities of both indicators of aerobic capacity measured (CS and CCO) increased in the brain of fish with increasing growth in length (Table 3), but not with increasing condition factor or growth in mass. Thus, brain aerobic capacity is apparently higher only when the rate of somatic biosynthesis is higher. Future investigations should consider biochemical correlates of growth in length as potential predictors of growth in fish.

Using biochemical correlates of growth to estimate growth rate

Combinations of variables were identified that explained much (80–83%) of the variability of growth rate in our experimental fish (Table 4). HSI was the most strongly related to growth rate and condition factor (Table 2) and hence was part of every model retained. The other useful variables in a model for predicting growth rate were those that were least related to each other and hence came from different tissues: water contents of muscle, liver, and brain. The only metabolic variable that came forth as useful in our models for estimating growth rate was the activity of CS in the intestine. In the other tissues examined, water contents and other variables (such as enzyme activities) varied together with growth rate. In the intestine, however, since water content did not change with growth rate, CS activity in this tissue made a significant contribution to both models in Table 4 (it increased by 2–4% the prediction of growth rate in length or in mass, respectively; see Results). Finally, our analyses indicate that optimal combinations of biochemical indicators can explain the variability of growth rate in length as accurately as that of growth rate in mass (Table 4) and better than that of condition.

An ideal model for estimating growth rate of fish should be built with variables that are strongly dependent on growth but largely unaffected by condition. We already know that the HSI is not a reliable indicator of growth, even though it is closely related to growth in studies such as this one. In cod, an elevated HSI indicates favourable recent feeding conditions that resulted in hepatic lipid accumulation and may be found in a fish that is beginning to fast and to lose mass. Our study shows that models excluding HSI still explain much of the variability of growth rate in juvenile cod. Further research such as that conducted by Dutil et al. (1998) will hopefully provide metabolic variables that vary with growth rate but are unaffected by condition. The estimation of cod growth in the wild using metabolic variables could be a promising tool in the monitoring of cod stocks.

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Vulnerability to predation and physiological stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*) experimentally infected with *Renibacterium salmoninarum*

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Abstract: We experimentally infected juvenile chinook salmon (*Oncorhynchus tshawytscha*) with *Renibacterium salmoninarum* (Rs), the causative agent of bacterial kidney disease (BKD), to examine the vulnerability to predation of fish with differing levels of Rs infection and assess physiological change during progression of the disease. Immersion challenges conducted during 1992 and 1994 produced fish with either a low to moderate (1992) or high (1994) infection level of Rs during the 14-week postchallenge rearing period. When equal numbers of treatment and unchallenged control fish were subjected to predation by either northern squawfish (*Ptychocheilus oregonensis*) or smallmouth bass (*Micropterus dolomieui*), Rs-challenged fish were eaten in significantly greater numbers than controls by nearly two to one. In 1994, we also sampled fish every 2 weeks after the challenge to determine some stressful effects of Rs infection. During disease progression in fish, plasma cortisol and lactate increased significantly whereas glucose decreased significantly. Our results indicate the role that BKD may play in predator–prey interactions, thus ascribing some ecological significance to this disease beyond that of direct pathogen-related mortality. In addition, the physiological changes observed in our fish during the chronic progression of BKD indicate that this disease is stressful, particularly during the later stages.

Résumé : Nous avons expérimentalement infecté des juvéniles de saumon quinnat (*Oncorhynchus tshawytscha*) par l'agent pathogène de la maladie bactérienne du rein (MBR), le *Renibacterium salmoninarum* (Rs), dans le but d'examiner la vulnérabilité à la prédation des poissons présentant des niveaux d'infection divers par Rs et d'évaluer leur évolution physiologique pendant la progression de la maladie. Des épreuves par immersion réalisées en 1992 et 1994 ont permis d'obtenir des poissons présentant un niveau d'infection faible ou moyen (1992) ou élevé (1994) par Rs pendant les 14 semaines suivant les épreuves. Des nombres égaux de poissons ayant subi l'épreuve et de poissons témoins ont été exposés à la prédation par des sauvageses du nord (*Ptychocheilus oregonensis*) ou des achigans à petite bouche (*Micropterus dolomieui*). Les poissons ayant subi l'épreuve au Rs ont été consommés en nombre significativement plus élevé que les témoins, par un facteur atteignant presque deux. En 1994, nous avons aussi prélevé des poissons à toutes les deux semaines après l'épreuve dans le but de déterminer certains effets découlant du stress de l'infection par Rs. Nous avons noté une augmentation significative du cortisol et du lactate plasmatiques et une baisse significative du glucose plasmatique pendant la progression de la maladie. Nos résultats montrent que la MBR peut influer sur les relations prédateur–proie. Cela donne une certaine importance écologique à la maladie, en plus de la mortalité découlant directement des effets du pathogène. En outre, les modifications physiologiques notées chez les poissons pendant la progression chronique de la maladie indiquent que celle-ci est source de stress, plus particulièrement pendant les dernières étapes.

[Traduit par la Rédaction]

Introduction

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum* (Rs), has long been a significant contributor to mortality of juvenile salmonids in hatcheries in the Columbia River basin. The disease is not only considered to be a major obstacle to the successful culture of salmonids, but is prevalent in and presumably has some impact on wild populations (e.g., Sanders et al. 1992). The disease causes chronic mortality during hatchery residency of juvenile salmonids, and mortality may be exacerbated when fish enter salt water (Fryer and Sanders 1981; Banner et al. 1983). Control of BKD has been difficult to achieve and, because large gaps persist in our knowledge of the infection (Elliott et al. 1989), this disease will continue to receive a considerable amount of research interest.

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Little is known about the effects that BKD may have on selected aspects of fish performance capacity, such as predator avoidance ability. In addition, there is scant — and equivocal — information on whether infection with Rs per se is stressful and thus activates the hypothalamic–pituitary–interrenal (HPI) axis and elicits physiological stress responses (Suzumoto et al. 1977; Donaldson 1981). Because the impact of BKD on migrating hatchery and wild fish has been difficult to evaluate (Sanders et al. 1992), examining the effects of BKD on ecologically relevant performance characteristics should at least partially elucidate the functional significance of BKD and the role that it plays in the survival of fish in the wild.

The recent development of more sensitive and quantitative detection methods for Rs antigen, such as the enzyme-linked immunosorbent assay (ELISA; Pascho and Mulcahy 1987), has allowed researchers and pathologists to more accurately determine the prevalence and level of severity of Rs infections in salmonid populations (Pascho et al. 1987). Consequently, fish have been qualitatively classified as having low, moderate, and high levels of Rs antigen, corresponding to infection severity, based on ELISA results (Pascho et al. 1991; Elliott et al. 1997). This implies that differing infection levels may have some adaptive significance, but thus far, such levels appear to be of clinical significance only. We attempt to ascribe some ecological significance to infections of Rs by examining the vulnerability to predation of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) with differing Rs infection levels and also assessing stress-related physiological changes in fish during the long-term, chronic progression of the disease.

Methods

We conducted two series of experiments to evaluate the predator avoidance ability of Rs-infected prey, one series in 1992 using northern squawfish (*Ptychocheilus oregonensis*) as predators and another in 1994 using smallmouth bass (*Micropterus dolomieui*) as predators. We also sampled Rs-infected prey for physiological assessment during disease progression, but only in 1994. Several aspects of our methods differed between years, as indicated subsequently.

Test fish

Juvenile subyearling spring chinook salmon were used for all experiments. All fish were offspring from adults with a negative or low Rs infection level as determined by ELISA segregation (Pascho et al. 1991). In both years, we obtained fish (1992: average length ± SE = 84.6 ± 1.4 mm, average weight = 6.7 ± 0.34 g, $N = 50$; 1994: average length = 101.8 ± 0.95 mm, average weight = 12.9 ± 0.38 g, $N = 40$) from Entiat National Fish Hatchery (Washington) and transported them to our laboratory using a truck with a large aluminum tank and aerated water of about 10°C. In 1992, upon arrival, about 750 fish were placed indoors into each of four 1.2-m-diameter circular tanks receiving 10 ± 1°C well water at a rate of about 4 L/min. Fish in two adjacent tanks were classified as controls and not challenged with Rs whereas fish in the other two tanks were quarantined and classified as Rs-challenged (treatment) groups. In 1994, we placed 250 fish into each of six 1.5-m-diameter tanks receiving 12 ± 1°C water: three tanks holding control fish and three tanks holding treatment fish. Volumes in all tanks were adjusted to yield low rearing densities that ranged from 7.5 to 9.5 g/L. Fish were fed twice daily with moist pellets (at a ration of about 3% body weight per day) and kept under an ambient photoperiod simulated with lights and timers that produced a gradual-intensity dusk and dawn. In 1992, all control fish were marked by clipping their adipose fin several weeks prior to the start of experiments. In 1994, we marked all treatment fish.

In 1992, we used six northern squawfish as predators, collected from the Columbia River by electrofishing. They averaged about 400 mm in fork length and were held indoors in a 7.6-m-long, 1.2-m-wide, 1.2-m-deep flowing water raceway maintained at 17 ± 1°C. In 1994, we used 14 smallmouth bass as predators, also collected from the Columbia River by electrofishing. They averaged 295 mm in length and seven fish were placed into each of two 3.75-m-diameter circular tanks receiving 15 ± 1°C well water. These tanks were 1 m deep and lined with gravel and cobble substrates with several pieces of 0.25-m-long, 15-cm-diameter PVC pipe randomly scattered along the bottom to serve as cover for the predators. All tanks were surrounded with curtains to minimize outside disturbance, and fish were held under a simulated ambient photoperiod. All predators were fed a maintenance diet of juvenile chinook salmon, and predation tests, which occurred in the same tanks, did not commence until predators were consistently feeding.

Bacterial immersion challenges

Treatment fish were experimentally infected with Rs using an immersion challenge procedure similar to those described by Murray et al. (1992) and Elliott and Pascho (1995). The bacterium used for the challenges was Rs, isolate DWK 90. A 1-mL aliquot of the isolate was grown in 1 L of KDM-2 broth media with serum at 15°C for 9 days. Log-phase cells were then harvested by centrifugation and resuspended in cold, sterile peptone–saline (0.1% w/v peptone + 0.85% NaCl). The concentration of Rs cells in the final suspension was determined by a membrane filtration – fluorescent antibody test, spectrophotometry, and culture on KDM-2 agar media.

The immersion challenge procedure differed slightly between years. In 1992, the water level in all four tanks was first lowered to 500 L by inserting new standpipes. Inflow water was shut off and oxygen from tanks and air from aquarium pumps was added to each tank via airstones. The two treatment tanks each received 1 L of broth culture containing about 1.9×10^9 bacteria/mL, thus yielding a challenge dose of about 3.8×10^6 bacteria/mL. Control tanks each received 1 L of sterile peptone–saline. The liquid was poured throughout the tank and the challenge continued for 24 h, after which time the water was partially drained from each tank, the original standpipes replaced, and flow reestablished. In 1994, fish in each tank were divided into two 108-L sterilized plastic buckets filled with 76 L of water from the tanks. The water level in the tanks was then lowered by inserting new standpipes and the buckets were then placed back into the tanks, which then served as water baths to help maintain temperatures during the challenge. We then poured 323 mL of broth containing 3×10^9 bacteria/mL into each bucket containing treatment fish, thus yielding a challenge dose of 1.27×10^7 bacteria/mL. Buckets containing control fish each received a similar aliquot of sterile peptone–saline. Lids were then placed on the buckets, and airstones connected to aquarium pumps were inserted through the lids and into the water. Again, challenges continued for 24 h before reinserting the original standpipes and liberating fish back into the tanks. The challenge in 1992 was conducted on 30 April and in 1994 on 1 June.

Holding and sampling

After the 24-h challenges had ended, fish were maintained as described above (at 12–13°C) for a period of at least 100 days. To monitor progression of the disease during the holding period and to estimate the time when predation trials should commence, in 1992, we necropsied 50 control and 50 treatment fish for analysis of whole kidneys by ELISA at 2, 6, 10, 12, 13, 14, and 15 weeks after the challenge ended. Twenty-five fish from each tank were rapidly removed by dip netting and placed in a lethal dose (200 mg/L) of tricaine methanesulfonate. After recording the length and weight of each fish, the kidney was removed aseptically and frozen at –80°C for future analysis by ELISA as described by Pascho and Mulcahy (1987) and modified by Elliott and Pascho (1991). In 1994, we sampled

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20 control and 20 treatment fish for kidneys and blood, just before the challenge and at 2, 4, 6, 8, 10, 12, and 14 weeks after the challenge. Fish were processed as previously described, except that blood samples were collected before removing kidneys by bleeding fish into ammonium-heparinized capillary tubes after severance of the caudal peduncle. Plasma was obtained by centrifugation and stored at –80°C for future analysis. Plasma cortisol was determined by enzyme immunoassay modified from procedures described by Munro and Stabenfeldt (1984). Plasma glucose and lactate were measured with a biochemistry analyzer (Yellow Springs Instruments, model 2700D).

Predation experiments

Predation experiments were composed of several replicate trials, conducted either in the raceway using northern squawfish or in the 3.75-m circular tanks using smallmouth bass. Trials in 1992 were conducted on consecutive days from 3 to 20 August which was during weeks 13, 14, and 15 after the Rs challenge whereas in 1994, we conducted trials from 17 August to 12 September (weeks 11–14). Trials in 1994 were not done on consecutive days because of the relatively low consumption and, presumably, digestion rates by smallmouth bass. To account for this, we allowed from 2 to 4 days between predation trials using smallmouth bass.

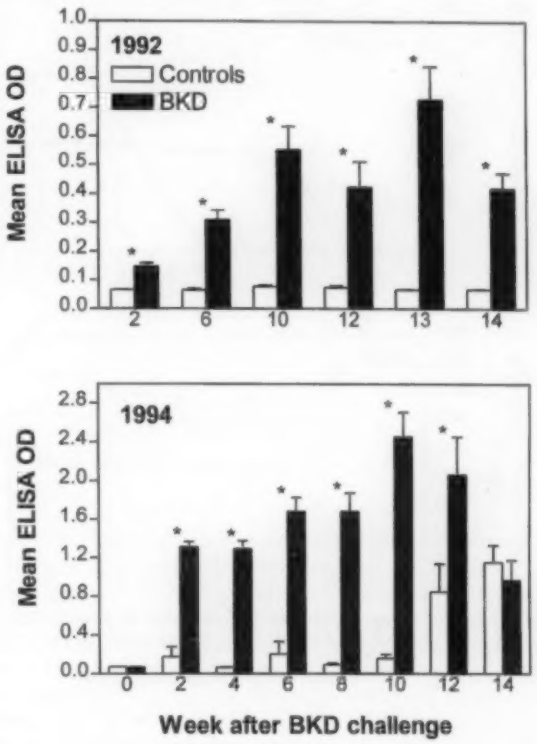
To begin a trial in 1992, 12 or 13 fish from each prey holding tank (i.e., 25 control and 25 treatment fish) were rapidly transferred at 08:00 into 19-L buckets filled with water and poured into the upstream end of the raceway. In 1994, only 10 control and 10 treatment fish were released to predators. In all cases, predation was allowed to continue for 24 h, at which time, all survivors were netted, identified as control or treatment fish, and measured. In 1992, we also excised the kidneys of survivors for analysis by ELISA to compare their severity of infection with that of our stock of control and treatment fish. We were able to observe the tests during daylight hours and made general behavioral observations, while concealed, from overhead.

Statistical analysis

Because there were no differences in average level of Rs infection as determined by ELISA absorbances between fish in replicate tanks on each sampling day (*t*-tests or ANOVA, $P > 0.05$), data were pooled for analysis. On each sampling day, we then compared the mean ELISA absorbances between control and treatment fish using a two-sample *t*-test and plotted the distribution of ELISA values. The positive–negative threshold absorbance values for the ELISA were calculated as described by Pascho et al. (1987). Fish with a positive Rs infection with absorbance between the positive–negative threshold and 0.199 were considered to have low infection levels, fish with absorbance between 0.200 and 0.999 were considered to have moderate infections, and those with absorbance >1.000 were considered to have high infection levels (Pascho et al. 1991, 1993). Mortalities during the holding period were tallied, plotted cumulatively, and sometimes necropsied to assess general physical condition. We compared the mean lengths of fish in the treatment and control groups on each sampling day using two-sample *t*-tests. For the physiological data on each sampling day, we also found no significant differences in average concentrations of cortisol, glucose, or lactate from fish in each tank and therefore pooled the data for analysis. For each sample day, we calculated overall mean concentrations for treatment and control fish and compared them using two-sample *t*-tests for use with equal or unequal variances. For all tests, the level of significance was 0.05.

Predation data were analyzed and statistical power considerations assessed in a manner identical to that of Mesa (1994). A heterogeneity chi-square analysis first determined that individual tests were homogeneous ($P > 0.250$; Sokal and Rohlf 1981). Chi-square goodness-of-fit tests were then used on pooled data to determine if predation was random (i.e., 50:50) on treated versus control fish.

Fig. 1. Mean (and SE) ELISA optical densities at selected time intervals after spring chinook salmon had received a waterborne challenge with *Renibacterium salmoninarum* and for unchallenged control fish during 1992 (upper panel) and 1994 (lower panel). Sample size was 50 fish per treatment in 1992 and 20 fish per treatment in 1994. Asterisks denote mean values within a time period that differ significantly ($P < 0.05$).



Results

The immersion challenges were successful, in both years, in producing fish with established infections of Rs in treatment fish (Fig. 1), although rate of progression and average level of infection severity differed. In 1992, mean ELISA absorbance rose gradually to about 0.6 during the first 10 weeks and maintained values of about 0.5–0.7 thereafter (Fig. 1). Mean ELISA absorbances of treatment fish were significantly ($P < 0.001$) higher than those of control fish for all sampling days. Control fish showed either very low or negative infection levels for the duration of the experiment. Most (87%) fish receiving the challenge had ELISA values corresponding to a low or moderate infection level. In 1994, mean ELISA absorbance increased rapidly in treatment fish to values >1.0 for all sample days except the last (Fig. 1). Also, control fish showed a significant, but unexpected, increase in average ELISA values during the last two sample days. Mean ELISA values were significantly ($P < 0.0001$) higher in treatment fish than in controls for all sample days except the first and last. Most (86%) treatment fish had ELISA values corresponding to a moderate or high infection level whereas most (84%) control fish had a negative or low infection level.

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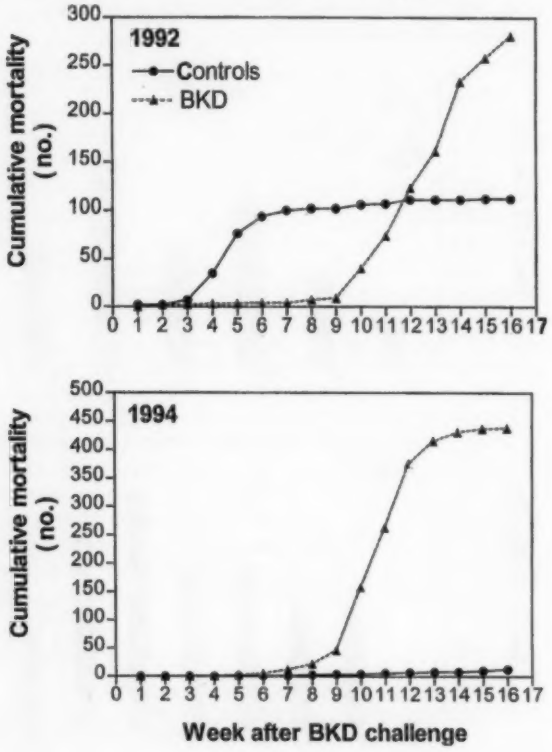
Fig. 2. Cumulative mortality of spring chinook salmon after receiving a waterborne challenge with *Renibacterium salmoninarum* and for unchallenged control fish during 1992 (upper panel) and 1994 (lower panel). Data represent only fish that died in rearing tanks during the period of disease progression.



In 1992, mortalities were low in both control tanks, except during weeks 4–6 when some minor unexpected (and unexplained) mortality occurred in one tank (Fig. 2). In the treatment tanks, fish mortalities were low until week 10 when they started to increase considerably. In 1994, mortalities were essentially nonexistent in control fish except for a few fish that died during the last 2 weeks of the experiment (Fig. 2). The timing of mortality observed for treatment fish was similar between the two years of study, but the fish in 1994 experienced 38% more total mortality than fish in 1992. In both years, treatment fish that died usually showed clinical signs of BKD, including swollen abdomens, ascites fluid, kidney lesions, and exopthalmia.

In both years, all fish surviving throughout the experiment fed well, grew, and were active. In 1992, treatment fish were significantly larger than control fish at all sample periods except the first (Fig. 3). In 1994, both groups of fish grew at similar rates for the first 8 weeks, at which time, control fish grew significantly faster than treatment fish (Fig. 3).

All three indicators of physiological stress showed significant changes in treatment fish as the disease progressed in 1994 (Fig. 4). Plasma cortisol was low and similar between both groups of fish during the first 4 weeks, started to increase at 6 weeks, and then increased significantly in treatment fish during weeks 8–12 before declining to control levels by week 14. Although plasma lactate showed an unexplained increase in both groups at week 4, the trend in plasma lactate after 6 weeks essentially mirrored that of cortisol except for not showing such a dramatic decrease at week 14. Plasma glucose was somewhat erratic in both groups, being similar between groups during the first 4 weeks before decreasing significantly in treatment fish during weeks 6–8. Concentrations of glucose in treatment fish then rose gradually to levels that were significantly higher than those in controls at week 14.

Treatment fish were significantly more vulnerable to predation than control fish in both years of our study. In 1992, we conducted a total of 18 replicate trials and released a total of 450 fish to northern squawfish. Predators consumed a total of 280 fish and on average (± SE) consumed 31% (± 2.0%) of the fish released during a trial. Of the 280 fish eaten, 181 (65%) were of the Rs-challenged group, which differed significantly from random (chi-square = 24.01, $P < 0.001$). In 1994, we conducted a total of 12 replicate trials and released a total of 240 fish to smallmouth bass. Predators ate a total of 115 fish and on average ate 48% (± 2.8%) of the fish released during a trial. Of the 115 fish eaten, 76 (66%) were Rs challenged, which also differed significantly from random (chi-square = 11.904, $P < 0.001$). In 1992, the Rs infection levels of fish that had survived a predation trial were similar to those observed in the general populations of treatment and control fish during the trials (data not shown).

Discussion

Our results indicate that fish infected with either moderate or high infection levels of Rs were significantly more vulnerable to predation by two species of fish with distinctly different predatory tactics, thus providing some evidence of the functional, ecological significance of BKD and the role that it plays in affecting the survival of fish in the wild. Also, our physiological data indicate that the HPI axis responds as the Rs infection progresses, thus providing new insight into the nature and potential effects of BKD as a chronic stressor. Collectively, our results indicate that the importance of BKD as a mortality factor may be significantly greater in toto than simply mortality directly attributed to the pathogen, which is prevalent in populations of salmon in the wild (Sanders et al. 1992).

The effects of disease on vulnerability to predation has received considerable attention in a variety of taxa; however, such studies have focused almost entirely on the effects of macroparasites. Most evidence indicates that parasites are often associated with altered host behaviors that lead to an increased susceptibility to predation (e.g., Herting and Witt 1967; Moore 1983; Hudson et al. 1992; Lafferty and Morris 1996). In contrast, Coble (1970) and Vaughan and Coble (1975) found no evidence that infestation with various ectoparasites increased the vulnerability of fathead minnow (*Pimephales promelas*), brook trout (*Salvelinus fontinalis*), or yellow perch (*Perca flavescens*) to predation. To our knowledge, our study and that of Herting and Witt (1967), who found that bluegill (*Lepomis macrochirus*) infected with *Flexibacter columnaris* showed an increased vulnerability to predation by bowfin (*Amia calva*), are the only studies that have dealt with the predator avoidance effects of piscine bacterial pathogens.

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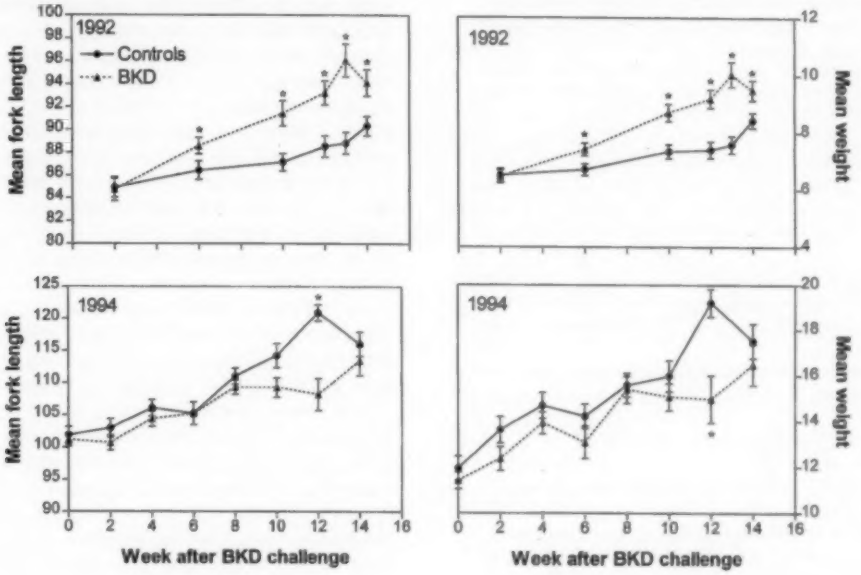
Fig. 3. Mean (± SE) fork lengths (mm) and weights (g) for spring chinook salmon that had received a waterborne challenge with *Renibacterium salmoninarum* and for unchallenged control fish during 1992 (upper panels) and 1994 (lower panels). Asterisks denote mean values within a time period that differ significantly ($P < 0.05$).



In many cases, predation is a key aspect of the life cycle of parasites because it allows the parasite to be transmitted from one host to another. However, such is not the case with Rs infections, since the disease is limited to the Salmonidae and, as far as we know, transmission occurs only horizontally or vertically and not by predation. Therefore, unlike many parasitic infestations, predation represents a substantial adaptive cost to the host *and* the bacteria.

The most often cited mechanism underlying increases in vulnerability to predation is alterations in behavior of the diseased organisms. Although changes in behavior could have played a role in the decreased predator avoidance ability of Rs-infected fish, we did not observe any overt differences in behavior between treatment and control fish. The decreased ability to avoid predators in our fish was likely due to the combined effects of behavioral and physiological performance changes as the disease progressed. For example, Giles (1983) and Sprengel and Lüchtenberg (1991) speculated that an increased vulnerability to predation was a probable consequence of the altered fright responses and swimming performance that they observed in their studies of parasitized fish. From a theoretical perspective, a potential, if not probable, reason for the decreased predator avoidance ability of Rs-infected fish is that such fish have a reduced metabolic scope for activity (sensu Fry 1947). We hypothesize that infection with Rs places considerable energetic demands on fish during the long, chronic progression of the disease and therefore leaves less energy available to perform other activities, such as predator avoidance. Evaluating the bioenergetic cost of disease may help elicit ultimate, rather than proximate, explanations for reduced performance in diseased organisms.

There were substantial differences in average ELISA values of treatment fish between years during the time when predation trials were done. Most fish in 1992 were classified as having a moderate infection level whereas most fish in 1994 had high infection levels. Low to moderate infection levels are commonly observed for various salmonids in the Columbia River (Maule et al. 1996) whereas fish with high infection levels are rarely seen, suggesting that our 1994 results may have little relevance to fish in the wild. Although it may not be too surprising that fish with high infection levels were more vulnerable to predation than were controls, our 1994 results may actually provide at least a partial explanation for *why* so few highly infected fish are found in the wild. Collectively, we believe that our results are ecologically relevant because the treatment effect was evident over a range of infection levels and two different types of predators produced similar results.

Although considerable evidence shows that various forms of environmental stress can predispose fish to disease (e.g., Snieszko 1974), less is known about whether the natural disease process per se constitutes a stressor in fish. Various pathogens have been shown to elicit a variety of general physiological responses in fish, including hyperglycemia and decreases in hematocrit, plasma osmolality, and plasma protein (e.g., Byrne et al. 1991; Haney et al. 1992; Grimnes and Jakobsen 1996). Although such physiological changes could be construed as being part of the stress response to disease, most studies of this type did not have the explicit objective of evaluating the stress responses to disease, nor were they necessarily designed to do so. Studies that have addressed the potential stress responses to disease have at least partially focused on the question of whether disease activates the HPI axis with the resultant secretion of cortisol, but results have been equivocal. For example, significant increases in plasma cortisol were not observed in studies dealing with rainbow trout (*Oncorhynchus mykiss*) infected with the blood parasite

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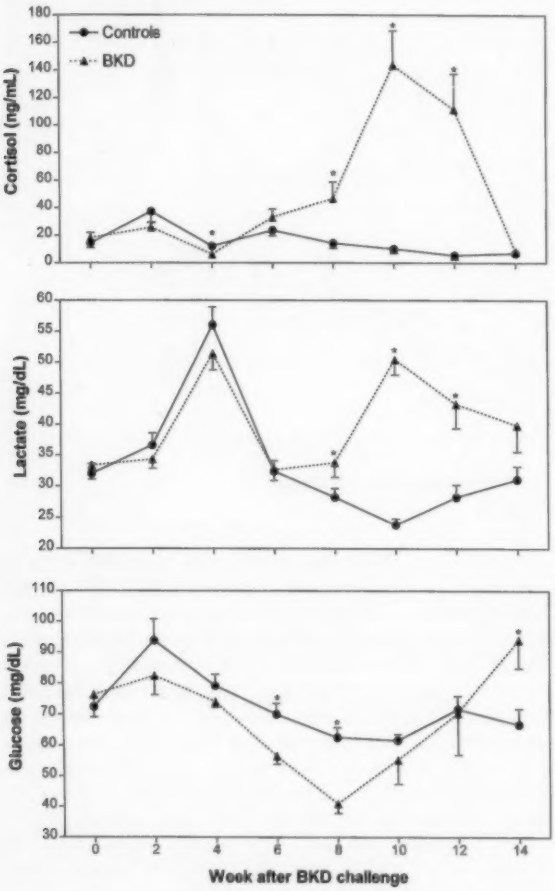
Fig. 4. Mean (and SE) concentrations of plasma cortisol (upper panel), lactate (middle panel), and glucose (lower panel) for spring chinook salmon that had received a waterborne challenge with *Renibacterium salmoninarum* and for unchallenged control fish during 1994. Asterisks denote mean values within a time period that differ significantly ($P < 0.05$).



Cryptobia salmositica (Laidley et al. 1988), in rainbow trout infected with *Ichthyophonus hoferi* (Rand and Cone 1990), or in chum salmon (*Oncorhynchus keta*) infected with erythrocytic necrosis virus (ENV; Haney et al. 1992). These findings led Laidley et al. (1988) and Rand and Cone (1990) to conclude that activation of the HPI axis and cortisol secretion may not be a normal component of the stress response to disease. In contrast, elevated plasma cortisol levels were observed in brown trout (*Salmo trutta*) infected with *Saprolegnia* (Pickering and Christie 1981), in red drum (*Sciaenops ocellatus*) infected with a bacterial infection (Robertson et al. 1987), and in Atlantic salmon (*Salmo salar*) having infectious salmon anaemia (ISA; Olsen et al. 1992).

Our results (and those from other as yet unpublished experiments in our laboratory) indicate that cortisol becomes substantially elevated only during the later stages of BKD, a time when ELISA values are high, moribund fish are common, and mortalities are rapidly increasing. To our knowledge, ours is only the third study reporting changes in plasma cortisol in salmonids infected with Rs, and the only study evaluating physiological changes in fish subjected to an immersion challenge with Rs. Donaldson (1981) discussed unpublished observations indicating that cortisol levels in salmonids were elevated due to BKD and that such levels may increase with an increasing severity of Rs infection. However, the opposite result was obtained by Suzumoto et al. (1977), who found that plasma cortisol may have decreased with the progressive development of BKD. Our results generally support those discussed by Donaldson (1981), except that we would describe cortisol elevations due to Rs infection as threshold, rather than progressive, responses. There are several possible reasons for the increase in plasma cortisol during the later stages of BKD, including stress associated with the apparent hypoxemia that exists in fish during this time, the secretion of interleukin-1or other cytokines by activated macrophages, stress associated with the general necrosis and breakdown of various organ systems, and a reduction in metabolic clearance of cortisol.

The trend in plasma lactic acid after 6 weeks in Rs-infected fish mirrored that of cortisol and suggests that fish were hypoxic. Lactate is an end product of glycolysis formed under anaerobic conditions and is a well-established indicator of stress due to fright or severe exertion (Wedemeyer et al. 1990). Just like cortisol, however, increases in lactate do not always occur as part of the stress response to disease. For example, Byrne et al. (1991) observed no increases in plasma lactate during the pathogenesis of bacterial gill disease in brook trout. Also, Haney et al. (1992) noted that lactate actually decreased in chum salmon infected with ENV. Given the rather severe hematological changes associated with BKD, we believe that the probable cause for elevated lactate is the same as that discussed by Olsen et al. (1992) for their fish, namely a hypoxemia resulting in poor oxygen transport. Furthermore, duration of elevated lactate in our fish was much longer than that typically observed in fish subjected to acute stressors. Long-lasting elevations in lactate were also reported by Olsen et al. (1992), and we concur with their observation that such responses must put serious demands on acid–base balance in fish.

Hyperglycemia is a classic response of organisms to a variety of types of stressors, yet the response of plasma glucose to disease is highly variable. Our results revealed a general decrease in plasma glucose through 8 weeks followed by a gradual increase during weeks 10–14. Depressed levels of plasma glucose in fish have been reported by others assessing the physiological effects of BKD (Wedemeyer and Ross 1973; Iwama et al. 1986) and cryptobiosis (Laidley et al. 1988). In contrast, Robertson et al. (1987) noted only mild elevations of glucose in red drum with a bacterial infection, and Rand and Cone (1990) and Haney et al. (1992) reported little change in glucose in fish infected with *I. hoferi* or ENV. Hypoglycemia in fish is thought to occur primarily because of inanition (Wedemeyer et al. 1990), but reduced food intake was probably not a major cause of low glucose levels in our fish, since they fed well throughout the experiment. We hypothesize that the reason for low plasma glucose in our Rs-infected fish was an excessive use of this energy substrate to help combat the infection. We hope our studies in progress on the metabolic cost of BKD will provide more insight into the dynamics of energy sources used by fish to help combat chronic, long-lasting infections.

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Fish diseases are an important force in structuring fish communities in the Pacific Northwest (Li et al. 1987), but a lack of data on the ecological impacts of disease has limited our understanding of the possible mechanisms behind this structuring force. Our results indicate the role that BKD may play in predator–prey interactions, thus ascribing some ecological significance to this disease beyond that of direct pathogen-related mortality only. In addition, the physiological changes observed in our fish during the chronic progression of BKD indicate that this disease is stressful, particularly during the later stages. More information is needed on the effects of exogenous stress and physiological changes in Rs-infected fish to further our understanding of the role that BKD may play in affecting the survival of fish in the wild.

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Method for estimating variability due to sampling of catches on a trawl survey

A.J.R. Cotter

Abstract: Many catches arising on trawl surveys of fish stocks are sampled for the measurement of fish lengths and the collection of otoliths for age determination, thereby increasing the variability of the estimated abundance indices, expressed as catch per hour by species and year-class. This paper demonstrates how catch-sampling variability can be assessed, using the English groundfish survey of the North Sea (EGFS) as an example for analysis. Unbiased estimators are derived for catch-sampling covariance matrices for numbers-at-length of a species by size category, by haul, and by sampling stratum and for numbers-at-age by stratum. The same statistical principles could be applied to derive appropriate estimators for other surveys having different catch-sampling procedures. A method is also put forward for estimating the relative importance of variability due to catch sampling and that due to the design of the survey, i.e., due to the number, location, and timing of fishing stations and the method of fishing. Analysis of a small set of EGFS data for haddock (*Melanogrammus aeglefinus*) is included for illustrative purposes. Implications for survey design and catch-sampling efficiency are briefly discussed.

Résumé : Bon nombre des prises des campagnes de relevé par chalutage dans des stocks de poisson sont échantillonnées en vue de la mesure des longueurs des poissons et du prélèvement des otolithes pour la détermination de l'âge, ce qui accroît la variabilité des indices d'abondance estimés, exprimés sous la forme des prises par heure par espèce et classe d'âge. Le présent article montre comment la variabilité de l'échantillonnage des prises peut être évaluée, l'analyse s'appuyant sur le relevé anglais des poissons de fond en mer du Nord. On a calculé des estimateurs non biaisés pour établir des matrices de covariance de l'échantillonnage des prises correspondant à l'effectif selon la longueur d'une espèce par catégorie de taille, par trait de chalut et par strate d'échantillonnage, et à l'effectif selon l'âge par strate. Les mêmes principes statistiques peuvent s'appliquer au calcul d'estimateurs appropriés pour d'autres campagnes ayant des procédures d'échantillonnage des prises différentes. Nous proposons aussi une méthode pour estimer l'importance relative de la variabilité due à l'échantillonnage des prises et de celle due aux paramètres de la campagne, soit le nombre, le lieu et le moment des opérations, ainsi que la méthode de pêche. Nous présentons à titre d'exemple l'analyse d'un petit ensemble de données du relevé anglais portant sur l'aiglefin (*Melanogrammus aeglefinus*). Enfin, nous traitons brièvement des implications de notre démarche en ce qui a trait à la conception des campagnes et à l'efficacité de l'échantillonnage des prises.

[Traduit par la Rédaction]

Introduction

Trawl surveys are used to estimate abundance indices for fish species in terms of numbers-at-age caught per unit of trawling time. The optimal numbers and locations of fishing stations and the best trawling methods to use have been widely considered (e.g., Doubleday and Rivard 1981; Smith and Gavaris 1993). Much less attention has been paid to another source of variability, that arising from the sampling of catches to obtain length frequency distributions and age–length keys (ALKs) (Ketchen 1949). The sampling scheme may change radically from haul to haul because of markedly different quantities, species, and size categories present, and the number of age determinations is often tightly limited by the skill and time required to do them. The statistical implications of catch sampling on trawl surveys have been recognized repeatedly (Westrheim 1967), but few analyses have been published. Better knowledge could lead to better methods on deck and ultimately to better surveys.

Statistical analyses are available for the related problem of sampling large-scale commercial landings of fish at markets (e.g., Gulland 1955; Doubleday and Rivard 1983; Lai 1993). However, these are of limited applicability to groundfish surveys for one or more of the following reasons: (1) variability of raising factors needed to give numbers-at-age from proportions-at-age is ignored, (2) small numbers in species groups and size categories, as occur in many catches on surveys, are not allowed for, (3) exact formulae available for the variance and covariance of products (Goodman 1960, 1962; Bohrnstedt and Goldberger 1969) are not used, and (4) dependences among estimated numbers in different length or age groups are ignored.

The last point is a simplification adopted by many fisheries studies, but positive or negative covariances should be expected among survey abundance indices for different species, age-classes, or length-classes because a trawl catch is a cluster, rather than a random sample of fish from the sea. The content of the cluster depends on the distribution and behaviour of the various species, the prevailing environmental conditions, and the manner of trawling. Taking a sample of n fish from a catch superimposes negative covariance because the more fish picked in one class, the fewer will have been picked from any

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other. Overlooking covariances among a set of estimated abundance indices can lead to the drawing of more inferences about stocks than are supported by the data, overfitted models, misleading error distributions, and other problems.

The present paper shows how unbiased estimators for the catch-sampling component of variances and covariances, together referred to as "dispersion," can be derived for a trawl survey, giving attention to the listed points. The approach consists of (*i*) setting out the equations used to estimate the abundance indices, (*ii*) applying standard rules of statistical expectation to each equation, and (*iii*) identifying variables that are sufficiently independent to permit simplifications. The population being sampled is taken as the total number of fish of one species arriving on the deck of the survey vessel at one or more stations. Limiting the analysis to a single species simplifies notation and keeps the dispersion matrices to manageable sizes.

The second component of survey dispersion arises from the choice of when, where, and how the catches were trawled and will be referred to as the "design" component. A method for assessing the relative importance of the catch-sampling and design components of dispersion is put forward for the case when catch-sampling procedures are independent of survey design. The estimator of design dispersion is unbiased if the set of catches is an independent random sample from all possible catches in the survey area, but is not if the survey itself is biased, e.g., if fixed station positions are revisited annually.

Many groundfish surveys are conducted around the world, and probably few use exactly the same catch-sampling procedures. The survey analysed here, the English groundfish survey of the North Sea (EGFS) (Anonymous 1992, 1996), is chosen for reasons of familiarity to the author, not because commendation or criticism is intended. The statistical equations derived are applied to a small set of EGFS data for haddock (*Melanogrammus aeglefinus*) for illustration, and general implications for trawl survey practices are discussed.

EGFS methods

The annual EGFS visits 74 fixed stations grouped into seven sampling strata known as the North Sea roundfish areas (RFA) stretching from the English Channel to the Shetland Islands. A standard trawl is towed for a standard time (Anonymous 1992) at each station. Catches are sorted variably according to quantity and content, the immediate need being to clear the facilities on deck in time to permit hauling at the next station. Usually, the whole catch is sorted into baskets by species and size categories, i.e., small, medium, and large, before weighing. If there are too many baskets to process, a sample of baskets is drawn randomly after first thoroughly shuffling the contents of all them (Gulland 1966). When a catch is extremely large, e.g., a tonne or more, all fish are basketed and weighed, but only every *n*th basket is removed for shuffling and sampling. Provided that the systematic sampling and the common raising factor for all size categories do not cause significant bias, no special considerations are raised.

A double-sampling scheme (Thompson 1992) is used. The first sample provides from 50 to a few hundred individuals of each species for length measurements. Otoliths for age determination are removed from a subsample of the measured fish and then aggregated across stations to form a single ALK for each RFA. A quotum of otoliths to be collected within the RFA is set for each centimetre length division. Regionalisation of the ALK in this way makes reasonable numbers of age determinations available for estimating proportions-at-age-given-length, but can lead to an uneven representation of stations, with sometimes the last to be fished being unsampled. The implicit assumption is that the length-at-age relationship is approximately constant within each RFA.

Notation and theory

For any single species, let s index the strata (or RFA) of the survey where $s = 1,..., S$; h index the hauls (catches), one per station, within a stratum where $h = 1,..., H_s$, c index the size categories within a catch where $c = 1,..., C_{h,s}$, l index the length divisions for measuring the fish, usually whole or half centimetres, where $l = 0,..., L$, and a index ages in years where $a = 0,..., A$. A second independently varying index for the same variable is distinguished by adding a prime.

Two rules of statistical estimation will be referred to by Roman numerals. Firstly, for random variables x and y, coefficients b and b', and indices $i, j = 1,..., n$:

$$\text{(I)} \quad \mathrm{Cov}\left(\sum_i b_i x_i, \sum_j b'_j y_j\right) = \sum_i \sum_j b_i b'_j \cdot \mathrm{Cov}(x_i, y_j).$$

An unbiased estimator is obtained by substituting unbiased covariance estimates on the right-hand side.

Secondly, for random variables x_1, x_2, y_1, and y_2, both x being independent of both y, an unbiased estimator for the covariance of products is

$$\text{(II)} \quad \mathrm{cov}(x_1y_1, x_2y_2) = x_1x_2 \cdot \mathrm{cov}(y_1, y_2) + y_1y_2 \cdot \mathrm{cov}(x_1, x_2) - \mathrm{cov}(x_1, x_2) \cdot \mathrm{cov}(y_1, y_2)$$

if both of the covariance estimators on the right-hand side are unbiased. See the Annex for the derivation of this formula. Because (xy) is itself a random variable and given $\mathrm{cov}(x_1y_1, x_2y_2)$ unbiased, eq. II can be applied iteratively to find the covariance of triple or higher products, (xyz), etc.

EGFS abundance indices

The following seven equations describe the abundance indices estimated from the EGFS. Let the true numbers-at-length of fish in the cth size category of a species in the hth haul taken from the sth stratum be $N_{l,c,h,s}$, or $N_{l,c}$ for brevity when h and s are implicit, and let the estimate of this obtained from a sample of n_c fish taken randomly for measurement from the size category be

$$\text{(1)} \quad \hat{N}_{l,c} = n_{l,c}\hat{W}_c\hat{w}_c^{-1}$$

where $n_{l,c}$ is the number of fish of length l in the sample, $\hat{W}_c$ is the measured weight (kilograms) of the species in the size category, and $\hat{w}_c$ is the measured weight of the sample. Size categorisation is generally a rapid, approximate process so that categories c and c', if adjacent, may both include fish of length l. The estimate for the haul is therefore

$$\text{(2)} \quad \hat{N}_{l,h} = \hat{N}_{l,c} + \hat{N}_{l,c'}$$

where either term on the right-hand side might be zero. These estimates are standardised for haul duration in hours, t_h (usually constant), and averaged over all hauls in the stratum to

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estimate mean numbers-at-length per station per hour for the stratum:

$$(3)\quad \hat{\bar{N}}_{l,s} = H_s^{-1} \sum_h \hat{N}_{l,h}/t_h.$$

For age determination, otoliths are taken from $y_{l,h}$ measured fish of length l in haul h and then aggregated over all hauls in the stratum to form a regionalised ALK. Let the estimated proportion-at-age-given-length for stratum s be

$$(4)\quad \hat{P}_{all,s} = y_{all,s}/y_{l,s}$$

where $y_{all,s}$ is estimated numbers-at-age-given-length and $y_{l,s} = \Sigma_h y_{l,h}$. The estimate of mean numbers-at-age-given-length per station per hour is then

$$(5)\quad \hat{\bar{N}}_{all,s} = \hat{\bar{N}}_{l,s} \hat{P}_{all,s}$$

and the required estimate of mean numbers-at-age used as the abundance index for the stratum is

$$(6)\quad \hat{\bar{N}}_{a,s} = \sum_l \hat{\bar{N}}_{all,s}.$$

Stratum estimates may be of value by themselves, but are usually also raised by stratum area, K_s, to estimate an abundance index for the whole survey region, area $K = \Sigma K_s$:

$$(7)\quad \hat{N}_a = K^{-1} \sum_s \hat{\bar{N}}_{a,s} K_s.$$

Estimating catch-sampling dispersion matrices

Weighing errors

The variance of the raising factor, $\hat{W}\hat{w}^{-1}$ in eq. 1, can be found from the variances of $\hat{W}$ and $\hat{w}^{-1}$ estimated from special, replicated weighing trials made at sea. Applying eq. II:

$$(8)\quad \mathrm{var}(\hat{W}\hat{w}^{-1}) = \hat{W}^2 \cdot \mathrm{var}(\hat{w}^{-1}) + \hat{w}^{-2} \cdot \mathrm{var}(\hat{W}) - \mathrm{var}(\hat{w}^{-1}) \cdot \mathrm{var}(\hat{W}).$$

The catch and sample weighings should be unbiased and independent.

Numbers-at-length

One size category

For simplicity, the possibilities for mistaken measurements and counts will be ignored, and variances and covariances of zero will be assumed for numbers-at-length when all fish in a size category (or uncategorised species group) are measured.

The probability of obtaining $\{n_0, n_1, \ldots, n_L\}$ fish in $L + 1$ length divisions after measuring a random sample of n from a size category containing N individuals is given by the multivariate hypergeometric distribution (MHD) (Johnson and Kotz 1968; Sukhatme and Sukhatme 1970). It is necessary to use $\hat{W}_c\hat{w}_c^{-1}$ as the raising factor for a size category c, instead of $N_c n_c^{-1}$, since N_c is unknown. Unbiased estimators relating to eq. 1 are

$$(9a)\quad \mathrm{var}\hat{N}_{l,c} = \hat{N}_{l,c}(\hat{N}_c - \hat{N}_{l,c})(\hat{N}_c - n_c)/(n_c - 1)\hat{N}_c$$

and

$$(9b)\quad \mathrm{cov}(\hat{N}_{l,c}\hat{N}_{l',c}) = -\hat{N}_{l,c}\hat{N}_{l',c}(\hat{N}_c - n_c)/(n_c - 1)\hat{N}_c.$$

The MHD becomes the multinomial distribution as the finite population correction, $(\hat{N}_c - n_c)/\hat{N}_c$, increases to unity. The correlation coefficient is

$$(9c)\quad \mathrm{corr}(\hat{N}_{l,c},\hat{N}_{l',c}) = -\{\hat{N}_{l,c}\hat{N}_{l',c}(\hat{N}_c - \hat{N}_{l,c})^{-1}(\hat{N}_{l,c} - \hat{N}_{l',c})^{-1}\}^{1/2}.$$

To allow for weighing variance, apply eq. II to eq. 1:

$$(10)\quad \mathrm{cov}^*(\hat{N}_{l,c},\hat{N}_{l',c}) = \hat{W}_c^2\hat{w}_c^{-2} \cdot \mathrm{cov}(n_{l,c},n_{l',c}) + n_{l,c}n_{l',c} \times \mathrm{var}(\hat{W}_c\hat{w}_c^{-1}) - \mathrm{cov}(n_{l,c},n_{l',c}) \cdot \mathrm{var}(\hat{W}_c\hat{w}_c^{-1})$$

where

$$\mathrm{cov}(n_{l,c},n_{l',c}) = \begin{cases} -n_{l,c}n_{l',c}(\hat{W}_c - \hat{w}_c)/\hat{W}_c(n_c - 1) & \text{for } l \neq l' \\ n_{l,c}(n_c - n_{l,c})(\hat{W}_c - \hat{w}_c)/\hat{W}_c(n_c - 1) & \text{for } l = l'. \end{cases}$$

The finite population correction is in terms of weights rather than the unknown N_c, and $\mathrm{var}(\hat{W}_c\hat{w}_c^{-1})$ comes from eq. 8.

Combining size categories within a haul

Covariances of numbers-at-length within a haul are obtained by applying eq. I to eq. 2 and noting that covariances with $c \neq c'$ are expected to be zero because categories are independently sampled. This gives

$$(11)\quad \mathrm{cov}(\hat{N}_{l,h},\hat{N}_{l',h}) = \mathrm{cov}(\hat{N}_{l,c},\hat{N}_{l',c}) + \mathrm{cov}(\hat{N}_{l,c'},\hat{N}_{l',c'}).$$

The covariances on the right-hand side come from eqs. 9*a* and 9*b* or eq. 10. When length divisions l and l' both occur in only one category, one of the terms on the right-hand side will be zero, and when in different categories, both will be zero.

Combining hauls within a stratum

It will be assumed that all haul durations, t_h, are known accurately for estimating $\hat{\bar{N}}_{l,s}$ with eq. 3 and that sample estimates of numbers-at-length are independent from haul to haul. An unbiased estimator of stratum covariance follows from eqs. 3 and I, given $t_h = t_{h'}$, and taking only the covariances within hauls to be nonzero:

$$(12)\quad \mathrm{cov}(\hat{\bar{N}}_{l,s},\hat{\bar{N}}_{l',s}) = H_s^{-2} \sum_h t_h^{-2} \cdot \mathrm{cov}(\hat{N}_{l,h},\hat{N}_{l',h}).$$

The individual haul covariances are obtained from eq. 11.

Proportions-at-age-given-length

Given that the selection of fish for length measurement at each station takes no account of age, and that spatial variability of length-at-age within the stratum is insignificant, a dispersion matrix for eq. 4 may be estimated by applying an MHD based on drawing the $y_{l,s}$ randomly from all $N_{l,s} = \Sigma_h N_{l,h,s}$ fish of length l caught in the whole RFA:

$$(13a)\quad \mathrm{var}(\hat{P}_{all,s}) = \hat{P}_{all,s}(1 - \hat{P}_{all,s})(\hat{N}_{l,s} - y_{l,s})/\hat{N}_{l,s}(y_{l,s} - 1)$$

and

$$(13b)\quad \mathrm{cov}(\hat{P}_{all,s},\hat{P}_{a'l,s}) = -\hat{P}_{all,s}\hat{P}_{a'l,s}(\hat{N}_{l,s} - y_{l,s})/\hat{N}_{l,s}(y_{l,s} - 1).$$

The correlation coefficient is

$$(13c)\quad \mathrm{corr}(\hat{P}_{all,s},\hat{P}_{a'l,s}) = -\{\hat{P}_{all,s}\hat{P}_{a'l,s}(1 - \hat{P}_{all,s})^{-1}(1 - \hat{P}_{a'l,s})^{-1}\}^{1/2}.$$

An allowance for weighing variability in the estimate of numbers-at-length for the stratum, $\hat{\bar{N}}_{l,s}$, is not given because the raising factor only affects the finite population correction term (cf. eqs. 9*a* and 9*b*).

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Numbers-at-age-given-length

The estimates of $N_{l,s}$ and $P_{all,s}$ used to evaluate $\hat{\hat{N}}_{all,s}$, eq. 5, can be assumed independent on the grounds that the numbers found to fall into a length division would not normally affect or be affected by the numbers from that division found to fall into different age groups. Applying eq. II to eq. 5 gives

$$(14)\quad \operatorname{cov}(\hat{\hat{N}}_{all,s},\hat{\hat{N}}_{a'l'r,s}) = \hat{\hat{N}}_l\hat{\hat{N}}_{l'}\cdot\operatorname{cov}(\hat{P}_{all},\hat{P}_{a'l'r}) + \hat{P}_{all}\hat{P}_{a'l'r}\cdot\operatorname{cov}(\hat{\hat{N}}_l,\hat{\hat{N}}_{l'}) - \operatorname{cov}(\hat{P}_{all},\hat{P}_{a'l'r})\cdot\operatorname{cov}(\hat{\hat{N}}_l,\hat{\hat{N}}_{l'}).$$

Equation 12 provides $\operatorname{cov}(\hat{\hat{N}}_l,\hat{\hat{N}}_{l'})$. Provided that age determinations in the different length groups are independent, $\operatorname{cov}(\hat{P}_{all},\hat{P}_{a'l'r}) = 0$ when $l \neq l'$. Equation 13 provides $\operatorname{cov}(\hat{P}_{all},\hat{P}_{a'l'r})$ when $l = l'$.

Numbers-at-age

Applying eq. I to eq. 6 gives

$$\operatorname{cov}(\hat{\hat{N}}_{a,s},\hat{\hat{N}}_{a',s}) = \sum_l\sum_{l'}\operatorname{cov}(\hat{\hat{N}}_{all},\hat{\hat{N}}_{a'l'r}).$$

Substituting eq. 14 with $\operatorname{cov}(\hat{P}_{all},\hat{P}_{a'l'r}) = 0$ when $l \neq l'$ and simplifying gives

$$(15)\quad \operatorname{cov}(\hat{\hat{N}}_{a,s},\hat{\hat{N}}_{a',s}) = \sum_l\{\hat{\hat{N}}_l^2 - \operatorname{var}(\hat{\hat{N}}_l)\}\cdot\operatorname{cov}(\hat{P}_{all},\hat{P}_{a'll}) + \sum_l\sum_{l'}\hat{P}_{all}\hat{P}_{a'l'r}\cdot\operatorname{cov}(\hat{\hat{N}}_l,\hat{\hat{N}}_{l'}).$$

Combining stratum estimates

Finally, the $\hat{\hat{N}}_{a,s}$ are raised by area to give estimates for the whole North Sea, as shown in eq. 7. Assume that $E[\operatorname{cov}(\hat{\hat{N}}_{a,s},\hat{\hat{N}}_{a',s'})] = 0$ for $s \neq s'$ by virtue of independent sampling of the catches in the different strata for both length and age. The catch-sampling covariance of the estimate of the North Sea abundance index is obtained by applying eq. I to eq. 7 and substituting eq. 15 on the right-hand side:

$$(16)\quad \operatorname{cov}(\hat{\hat{N}}_a,\hat{\hat{N}}_{a'}) = K^{-2}\sum_s K_s^2\cdot\operatorname{cov}(\hat{\hat{N}}_{a,s},\hat{\hat{N}}_{a',s})$$

where the measurements of area, K_s, are assumed accurate.

Removing catch-sampling variability

In this section, a method of estimating the design component of dispersion for each stratum is suggested. The stratum estimates could be combined to cover the whole survey area as for eq. 16 if required. The method is likely to be of most value when data for large numbers of stations are available because negative estimates of variance and correlations of magnitude greater than 1 can arise when the requisite dispersion matrices are poorly known. Searle et al. (1992) discussed the general problem of negative estimates of variance components.

Let

$$\hat{\hat{N}}_{a,s} = \eta_{a,s} + d_{a,s} + e_{a,s}$$

where $\eta_{a,s}$ is the true abundance index for fish of age a in the stratum, $d_{a,s} = \bar{\hat{N}}_{a,s} - \eta_{a,s}$ is the design error for the stratum, and $e_{a,s} = \hat{\hat{N}}_{a,s} - \bar{\hat{N}}_{a,s}$ is the catch-sampling error, all values being standardised per station per hour. Also, assume that $E(e_{a,s}) = E(d_{a,s}) = E(e_{a,s}d_{a',s})$ for all a and a'. Writing $d_a = x_1$, $e_a = x_2$, $d_{a'} = y_1$, and $e_{a'} = y_2$ in eq. I and substituting unbiased estimators for all terms with nonzero expectations gives

$$(17)\quad \hat{\mathbf{D}}_{d+e} = \hat{\mathbf{D}}_d + \hat{\mathbf{D}}_e$$

where $\hat{\mathbf{D}}_{d+e}$ is the product-moment dispersion matrix for $\hat{\hat{N}}_{a,s}$, $\hat{\mathbf{D}}_d$ is the dispersion matrix whose elements are $\operatorname{cov}(d_a,d_{a'})$, and $\hat{\mathbf{D}}_e$ is the dispersion matrix whose elements are $\operatorname{cov}(e_a,e_{a'})$, given by eq. 15. These are referred to as the total survey, the design, and the catch-sampling dispersion matrices, respectively. Design dispersion can therefore be estimated by subtraction of catch-sampling dispersion from total survey dispersion.

A complication arises when a single ALK is used for the stratum (as on the EGFS) because only one value is obtained for $\hat{\hat{N}}_{a,s}$ and $\hat{\hat{N}}_{a',s}$, so that the covariances in $\hat{\mathbf{D}}_{d+e}$ cannot be estimated. Instead, if there is more than one station in the stratum, a special ALK can be formed for each station. Then, $\hat{\mathbf{D}}_{d+e}$ can be estimated from numbers-at-age per station per hour, denoted $\hat{N}_{a,h}$.

Let

$$\tilde{\hat{N}}_{a,s} = H_s^{-1}\sum_h \hat{N}_{a,h}/t_h.$$

Then, from eq. I, after substituting estimators:

$$\operatorname{cov}(\tilde{\hat{N}}_{a,s},\tilde{\hat{N}}_{a',s}) = H_s^{-2}\sum_h\sum_{h'} r_h^{-2}\cdot\operatorname{cov}(\hat{N}_{a,h},\hat{N}_{a',h'}).$$

Using

$$\operatorname{cov}(\hat{N}_{a,h},\hat{N}_{a',h'}) = \left[\sum_h \hat{N}_{a,h}\hat{N}_{a',h} - H_s^{-1}\sum_h \hat{N}_{a,h}\sum_h \hat{N}_{a',h}\right]\times(H_S-1)^{-1}$$

for $h = h'$, and zero for $h \neq h'$ given that hauls are independent, the elements of $\hat{\mathbf{D}}_{d+e}$ become

$$(18)\quad \operatorname{cov}(\tilde{\hat{N}}_{a,s},\tilde{\hat{N}}_{a',s}) = H_s^{-2}\sum_h r_h^{-2}\cdot\operatorname{cov}(\hat{N}_{a,h},\hat{N}_{a',h}).$$

Example

The following is an analysis of a small set of data drawn arbitrarily from the August–September EGFS of 1990. The data are for haddock in RFA 3, an area of the North Sea spreading east of Scotland and representing about 12% of the total survey area. All trawl tows were 1 h, except at station 214 where the tow was 40 min for operational reasons.

Variable sampling and size categorisation practices are evident in Table 1, which shows category and sample weights for the 10 stations trawled together with raising factors and CVs estimated using eq. 8. Estimates of the variances of the individual weighings and their reciprocals given in the caption to Table 1 for both bench- and deck-mounted balances were derived from special replicated weighing trials conducted on the 1992 EGFS with balances and conditions similar to 1990. The variances of the category weights were calculated by multiplying the appropriate trial estimate by the number of weighings thought to have been made, assuming independence. CVs for raising factors greater than 1 varied between 0.5 and 4.8%.

Table 2 shows the numbers-at-length in catch samplings by size category ($n_{l,c}$) found by measuring samples from the various size categories at each station. The dispersion matrices for estimated numbers-at-length in the size category ($\hat{N}_{l,c}$) both

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Table 1. Analysis of estimated raising factors for haddock in various size categories from 10 trawl stations assuming independent weighings (kg) with variances as found in trials: B (bench balance), $\text{var}(\hat{W}) = 0.60 \times 10^{-5}$, $\text{var}(\hat{w}^{-1}) = 0.16 \times 10^{-3}$; D (deck balance), $\text{var}(\hat{W}) = 0.02$, $\text{var}(\hat{w}^{-1}) = 0.11 \times 10^{-6}$. EGFS 1990, RFA 3.

Station	Category	Category weight $\hat{W}_c$	Estimated no. of weighings and balance	Sample weight $\hat{w}_c$ and balance	Raising factor $\hat{W}_c\hat{w}_c^{-1}$	CV of raising factor (%)
169	1	6.910	1, B	—	1.00	—
172	1	8.264	1, B	—	1.00	—
	2	138.4	4, D	32.5, D	4.26	1.1
	3	34.0	1, D	13.7, D	2.48	0.6
	4	4.812	1, B	1.636, B	2.94	2.1
175	1	8.9	1, D	1.800, B	4.94	2.8
	2	97.5	3, D	13.7, D	7.12	0.5
	3	7.9	1, D	—	1.00	—
181	1	8.134	2, B	—	1.00	—
184	1	6.200	1, B	—	1.00	—
	2	48.6	2, D	24.5, D	1.98	0.9
	3	18.0	1, D	3.762, B	4.78	4.8
190	1	5.372	1, B	—	1.00	—
	2	56.7	2, D	12.6, D	4.50	0.6
	3	13.6	1, D	1.762, B	7.72	2.5
211	1	15.666	2, B	—	1.00	—
	2	5.144	1, B	1.570, B	3.28	2.0
214	1	6.3	1, D	—	1.00	—
	2	24.4	1, D	11.2, D	2.18	0.7
	3	7.3	1, D	1.336, B	5.46	2.6
215	1	24.472	?	—	1.00	—
	2	37.0	1, D	2.900, B	12.76	3.7
218	1	0.178	1, B	—	1.00	—
	2	13.1	1, D	2.525, B	5.19	3.4

Note: Sample weights were obtained with one weighing.

with and without weighing variance (eq. 9 or 10) were compared for category 3 of station 184, since this example displayed the largest CV (4.8%) for its sample raising factor (Table 1). The largest increase of variance for estimated numbers of fish due to weighing was 16% at 15 cm, while the most extreme reduction in the magnitude of the correlation coefficient was from –0.30 to –0.15 between the 14- and 15-cm divisions.

Table 3 shows estimated numbers-at-length by haul ($\hat{N}_{l,h}$) (eq. 2) rounded to integers and standardised per hour of trawling for each station. Dispersion matrices for each station (not shown) revealed high CVs for the less numerous length divisions, e.g., 93% for 38 cm at station 172 with only four fish per hour (Table 3). Elsewhere, CVs of 10–30% were typical. The largest negative correlation was –0.45 between 14 and 15 cm at station 175. The dispersion matrix for the stratum (not shown) provided the CVs for estimated total numbers-at-length caught in the stratum, $\hat{N}_{l,s}$, given in Table 3. The relationship between CVs and numbers caught was evidently affected by varying catch-sampling practices at the different stations; e.g., compare CVs and numbers caught at 18, 19, and 20 cm and note several CVs of 0% for length divisions with a raising factor of 1 at all stations, e.g., at 7 cm. The largest magnitude correlation for the stratum was –0.25 between lengths 14 and 15 cm when weighing variances were omitted from calculations and –0.21 when they were included.

Age determinations for samples of the measured haddock are shown in Table 4. Examples of dispersion matrices and correlations for proportions-at-age-given-length for the stratum ($\hat{P}_{all,s}$) estimated using eqs. 13*a* and 13*b* are shown in Table 5. Large-magnitude correlations were frequent, and perfect negative correlation occurred whenever there were only two age groups represented, e.g., 31 cm.

The final abundance indices, estimated as mean numbers per station per hour in the stratum ($\hat{N}_{a,s}$), and associated dispersion matrices and correlations are shown in Table 6, with and without weighing errors included. A relatively large CV, 55%, was found for 3-year-olds, being based on only three age determinations (see Table 4). Strong negative correlations are evident, ranging to –0.55 for fish of 1 year and older. These age groups displayed identical CVs after rounding to integers whether or not weighing variances were included in the calculations. The variance of numbers of age 0 fish, however, increased sharply when weighing variance was included, from 15.48 to 48.57, leading to a doubling of the CV, although still only 2%. The negative correlation between numbers of 0- and 1-year-olds almost halved from –0.67 without weighing variance to –0.36 with it.

To assess the relative importance of catch-sampling and design variability, numbers-at-age by haul ($\hat{N}_{a,h}$) were specially estimated by multiplying $\hat{N}_{l,h}$ (Table 3) by proportion-at-age-given-length by haul, $\hat{P}_{all,h}$, obtained using otoliths collected at one station only; the few fish over 4 years old were omitted. Results are given in Table 7*a*. At most stations, only one age determination was made for each centimetre length division. Nevertheless, the means for the stratum ($\bar{N}_{a,s}$), also shown in Table 7*a*, are approximately the same as the means ($\bar{N}_{a,s}$) in Table 6. The total survey dispersion ($\hat{D}_{d+e}$) was

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Table 2. Numbers-at-length, $n_{l,c}$, in samples from the size-categorized catches of haddock taken at 10 stations, EGFS 1990, RFA 3.

		Length (cm)																					
Station	Category	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
169	1				1	6	7	9	5	2			1								1		
172	1																						
	2																						
	3															1		6	14	16	16	11	5
	4			2		5	10	14	19	9	5												
175	1					1	5	4	13	28	6	3											
	2												1	2		2	8	15	18	7	6	3	3
	3																						
181	1	1			9	13	11	12	2								2	1	7	3	4	2	1
184	1																						
	2													1	6	4	13	35	24	15	20	8	11
	3			3	9	10	17	14	28	40	20	4											
190	1																						
	2											1	2	1	2	4	14	18	15	6	6	9	5
	3		2	1	10	20	12	11	21	7	5	1											
211	1											2	2	2		1		2	4	13	22	25	12
	2				1	5	12	19	13	5	6	3											
214	1																						
	2											1				1	3	6	10	8	12	11	6
	3			1	1	10	13	13	6	7	7	1											
215	1															1		1	6	4	22	32	19
	2				1	2	6	17	20	17	12	8	5										
218	1																				1		
	2						3	4	5	10	14	20	4	1									

Note: "7 cm" includes fish from 7 to <8 cm in length, etc. See Table 1 for raising factors.

Table 3. Estimated total numbers-at-length per hour, $\hat{N}_{l,h}$, in the catches of haddock taken at 10 stations, together with marginal sums, $\hat{N}_h$ and $\hat{N}_{l,s}$, and catch-sampling CVs (%) for $\hat{N}_{l,s}$ including weighing variance, EGFS 1990, RFA 3.

	Length (cm)																					
Station	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
169				1	6	7	9	5	2			1								1		
172			6		15	29	41	56	26	15					2		15	35	40	40	27	12
175					5	25	20	64	138	30	15	7	14		14	57	107	128	50	43	21	21
181	1			9	13	11	12	2								2	1	7	3	4	2	1
184			14	43	48	81	67	134	191	96	19		2	12	8	26	69	48	30	40	16	22
190		15	8	77	154	93	85	162	54	39	12	9	5	9	18	63	81	68	27	27	41	23
211				3	16	39	62	43	16	20	12	2	2		1		2	4	13	22	25	12
214			8	8	82	107	107	49	57	57	11				3	10	20	33	26	39	36	20
215				13	26	77	217	255	217	153	102	64			1		1	6	4	22	32	19
218						16	21	26	52	73	104	21	5							1		
$\hat{N}_{l,s}$	1	15	36	154	365	485	641	796	753	483	275	104	28	21	47	158	296	329	193	239	200	130
CV	0	68	33	18	11	10	9	8	8	11	15	28	40	31	27	15	10	9	11	9	9	12

computed with eq. 18 using only nine hauls ($H_s = 9$), since station 175 furnished no otoliths (see Table 7*b*). The results of applying eq. 17 to the dispersion matrices in Tables 7*b* and 6*b* are shown in Table 7*c* with associated correlations and CVs. One small-magnitude variance estimate was negative, that for age 3 fish, so that correlations for 3-year-olds could not be evaluated, and certain other correlations exceeded 1.0 in magnitude.

Discussion

The provision of more resources to take larger samples of

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Length (cm)																						
29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	n_c
2	2	3	1	1	2	1	1		1	3												49
										2	3	1	2	1			1				1	11
1	6	4	15	13	14	9	4	9	1	2	2											80
4	3	2																				78
																						64
																						60
4	4	3	3	3	2																	84
					3		2	2	3	1		1		1	1							14
1	1	5	1	2	1		1	1														81
				2	4	2		1		2		1					1					13
7	4	4	1	1			1															155
																						145
		3	2	5	3		2															15
2	3	1																				89
																						90
2							1															88
																						64
			2	2	5	5	3	1														18
4	2	5																				69
																						59
12	3	1	1	1	1	2	1					1						1				109
																						88
																						1
																						61

Length (cm)																						
29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	$\hat{N}_h$
2	2	3	1	1	2	1	1		1	3												49
14	33	22	64	55	60	38	17	38	4	11	12	1	2	1			1				1	733
28	28	21	21	21	17		2	2	3	1		1		1	1							906
1	1	5	1	2	1		1	1														81
14	8	8	2	4	4	2	2	1		2		1					1					1015
9	14	8	2	5	3		2															1113
2							1															297
13	7	16	3	3	8	8	5	2														738
12	3	1	1	1	1	2	1					1						1				1233
																						319
95	96	84	95	92	96	51	32	44	8	17	12	4	2	2	1	0	2	1	0	0	1	6484
17	19	18	18	18	16	21	23	24	47	31	44	0	0	0	0	0	0	0	0	0	0	

the catches, particularly for age determinations, may provide a low-cost way of improving the precision of a groundfish survey if catch sampling is the cause of a relatively large proportion of total variability. Alternatively, if it is not, there may be advantage to taking smaller samples so that more stations can be fished in the time available. Shorter towing times, as suggested by Pennington and Vølstad (1991), would assist this and would produce generally smaller catches needing less sampling, at least for length measurements. However, other practical matters have to be considered. Certain trawls, such as that used on the EGFS, require time on the bottom to stabilise in form depending on warp length and depth, and this

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Table 4. Numbers of measured haddock removed for age determination, $y_{l,s}$, and numbers-at-age, $y_{al,s}$, EGFS 1990, RFA 3.

Length (cm)	Age determinations	Age (years)									
		0	1	2	3	4	5	6	7	8	9
7	1	1									
8	1	1									
9	4	4									
10	7	7									
11	8	8									
12	9	9									
13	9	9									
14	9	9									
15	8	8									
16	7	7									
17	8	7	1								
18	5	4	1								
19	4	2	2								
20	2		2								
21	5		4	1							
22	4		4								
23	7		7								
24	7		7								
25	7		7								
26	9		8	1							
27	7		7								
28	7		2	5							
29	8		3	4		1					
30	7		1	5	1						
31	7		2	5							
32	7			4	1	2					
33	7			3	1	3					
34	7			5		2					
35	5			2		3					
36	8			3		5					
37	4			1		3					
38	2			1		1					
39	3					2		1			
40	1					1					
41	3					3					
42	1					1					
43	1					1					
44											
45											
46	2					2					
47	1										1
48											
49											
50	1					1					
Total	210	76	58	40	3	31	0	1	0	0	1

potential source of variability would increase in importance if the standard towing time were reduced. On the EGFS, shorter tows would not permit more stations to be fished because of the time taken to shoot and haul, the large geographic distances to be covered, and the restriction of fishing to daylight (Anonymous 1992).

Analyses of a wide spread of data for all species of interest should be undertaken before changes to a survey are contemplated. In the example, catch-sampling variability was estimated to be particularly important relative to design variability for the 2-year-old haddock (compare variances in Tables 6*b* and 7*c*), but this result is intended only to be illustrative, given that data from only nine stations were used. It should be remembered that the catch-sampling formulae presented here do not allow for inaccurate length or age measurements, poor counting, or poorly randomised sampling, should they occur. In addition, when using regionalised ALKs, as on the EGFS, no allowance is made for spatial variation of length-at-age within the region. Smith et al. (1992) reported occurrence of this for Pacific hake (*Meluccius productus*).

The estimator for the covariances of numbers-at-age for the stratum (eq. 15) embodies the effects of the age and length sample sizes and the age–length structure of the population of fish caught. If the lengths of all fish caught in the stratum were measured, $\text{var}(\hat{N}_l)$ and $\text{cov}(\hat{N}_l,\hat{N}_{l'})$ would become zero and

$$(19)\quad \text{cov}(\hat{N}_{a,s},\hat{N}_{a',s}) = \sum_l \hat{N}_l^2 \cdot \text{cov}(\hat{P}_{al},\hat{P}_{a'l}).$$

Dispersion of numbers-at-age, then, depends strongly on the covariance term and thus on y_l, the numbers of age determinations in each length division (see eq. 13).

Alternatively, if the catches were sampled for length measurement, but age determinations were made on all measured fish, $\text{cov}(\hat{P}_{al},\hat{P}_{a'l})$ is at the minimum for the given $\hat{N}_l$ and y_l, $\text{var}(\hat{N}_l)$ and $\text{cov}(\hat{N}_l,\hat{N}_{l'})$ are nonzero, and the age–length structure of the population expressed as $\hat{P}_{al}\hat{P}_{a'l'}$ has a maximal influence on dispersion.

The efficiency of catch sampling depends on the criterion used. Kimura (1977) proposed minimising $\Sigma\text{var}(\hat{P}_a)$, the equivalent objective function in the present context being $\Sigma\text{var}(\hat{N}_{a,s})$ (i.e., eq. 15 or 18 with $a = a'$). Jinn et al. (1987) and Lai (1993) generalised this to allow different relative weights for the variances and incorporation of covariances. Gulland (1955) proposed that the CVs of $\hat{N}_a$ should be approximately equal for all a. In the usual situation, many more lengths are measured than ages determined, so that eq. 19 is approximately true. This suggests that taking otoliths in proportion to $\hat{N}_l^2$ is the "rule of thumb" for decreasing $\Sigma\text{var}(\hat{N}_{a,s})$, while taking a fixed quotum in each length division is better for achieving constant CVs. Since the fixed-quota scheme was used on the 1990 EGFS, it should be noted that high CVs for 3- and 6-year-old fish in Table 6 resulted from the scarcity of those age groups (see Table 4).

In practice, quota sampling on the EGFS is a simple, dependable method of obtaining manageable numbers of otoliths for regional ALKs, provided that adequate fish at each age are caught. Making detailed efforts in advance of a survey to adjust quota in various length divisions to counteract the expected scarcity of one or more age groups would complicate the work at sea, leading to a greater chance of mistakes. It could also result in too much sampling effort being devoted to the least numerous age groups.

Quota need not, however, be completely flat over all length divisions. For example, selecting a gradual, stepped increase in the number of otoliths taken per length division to compensate for the increasing uncertainty about age-given-length with increasing size of fish would not be unduly complicated to implement. Firstly, note that the trace of the dispersion matrix for proportions-at-age-given-length (eqs. 13*a* and 13*b*) is proportional to $\Sigma\hat{P}_{al}(1 - \hat{P}_{al})$, summation being over all ages in

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Table 5. Three examples of catch-sampling dispersion matrices for proportions-at-age-given-length (with correlations substituted in the lower left triangle) and associated CVs (%) for haddock, EGFS 1990, RFA 3.

$l = 29$ cm, $y_{l,s} = 8$					$l = 30$ cm, $y_{l,s} = 7$				$l = 31$ cm, $y_{l,s} = 7$		
Age:	1	2	3	4	Age:	1	2	3	Age:	1	2
Proportions											
	0.375	0.5	0.0	0.125		0.143	0.714	0.143		0.286	0.714
Dispersion matrices and correlations											
Age					Age				Age		
1	0.031	−0.025	0.0	−0.006	1	0.019	−0.016	−0.003	1	0.031	−0.031
2	−0.780	0.033	0.0	−0.008	2	−0.650	0.032	−0.016	2	−1.000	0.031
3	0.0	0.0	0.0	0.0	3	−0.160	−0.650	0.019			
4	−0.290	−0.370	0.0	0.014							
CV	47	36	0	95		96	25	96		62	25

Table 6. Estimated mean numbers-at-age of haddock caught per station per hour, $\hat{N}_{a,s}$, catch-sampling dispersion matrices (with correlations in lower left triangle), and CVs (%) with (*a*) weighing variances excluded and (*b*) weighing variances included, EGFS 1990, RFA 3.

Estimated mean nos. per station per hour										
	Age (years)									
	0	1	2	3	4	5	6	7	8	9
	406.7	162.7	51.5	4.0	22.8	0	0.6	0	0	0.1
Dispersion matrices and correlations										
	(*a*) Excluding weighing variances									
Age										
0	15.48	−15.48								
1	−0.67	34.81	−18.51	−0.28	−0.54					
2		−0.55	32.56	−3.27	−10.78					
3		−0.02	−0.26	4.88	−1.32					
4		−0.03	−0.53	−0.17	12.88		−0.24			
5										
6					−0.14		0.24			
CV	1	4	11	55	16		82			
	(*b*) Including weighing variances									
0	48.57	−15.05								
1	−0.36	35.07	−18.50	−0.28	−0.54					
2		−0.55	32.62	−3.27	−10.75					
3		−0.02	−0.26	4.88	−1.32					
4		−0.03	−0.52	−0.17	12.91		−0.24			
5										
6					−0.14		0.24			
CV	2	4	11	55	16		82			

the length division, denoted A_l. Then, assuming that $\hat{P}_{a|l} \approx 1/A_l$ for all a, each length division with one age group will have a trace proportional to 0, with two age groups will have a trace proportional to 0.5, with three age groups will have a trace proportional to 0.67, with four age groups will have a trace proportional to 0.75, etc. The number of age groups in each length division can be estimated approximately from a suitable ALK and the available age determinations allocated to each division in proportion to the trace. In Table 4, for example, one might assume that one age only is present from 7 to 16 cm, two from 17 to 27 cm, three from 28 to 34 cm, and four from 35 cm upwards, the last ranges being very approximate. The required sum over all lengths is $(10 \times 0 + 11 \times 0.5 + 7 \times 0.67 + 16 \times 0.75) = 22.19$, and the 210 age determinations would be allocated to each length division at the rate of 9.5 per unit of this sum. This gives zero determinations per centimetre for 7–16 cm, five for 17–27 cm, six for 28–34 cm, and seven for 35 cm upwards, a simple stepwise progression with length. The last allocation would often be constrained by the lack of large fish (see Table 3), so a practical strategy would be to take otoliths from all fish over, say, 40 cm.

The EGFS practice of using separate ALKs for each RFA allows for variation of length-at-age between adjacent regions, but not within them. This is a compromise between drawing

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Table 7. (*a*) Numbers-at-age per station per hour, $\hat{N}_{a,h}$, for haddock 0–4 years old, estimated using otoliths collected at that station only (no otoliths were collected at station 175), (*b*) product-moment covariance matrix, $\hat{\mathbf{D}}_{d+e}$, for the stratum means, $\bar{\hat{N}}_{a,s}$ (with correlations in lower left triangle), and CVs (%) representing total survey variability, and (*c*) estimated design-related dispersion matrix, $\hat{\mathbf{D}}_d$ (with correlations in the lower left triangle), and CVs, EGFS 1990, RFA 3.

(*a*) Numbers-at-age per station per hour

	Age (years)					
Station	0	1	2	3	4	Total
169	31	1	7		10	49
172	188	159	195		191	733
181	48	19	10	2	2	81
184	694	212	98		11	1015
190	693	371	42	5	2	1113
211	215	80	1		1	297
214	487	188	63			738
215	1125	81	25		2	1233
218	318	1				319
Total						5578
Mean	422	124	49	1	24	

(*b*) Product-moment covariance matrix

Age					
0	1614	256	11.4	0.9	–69
1	0.47	184	42	1.6	10
2	0.04	0.43	50	–0.17	43
3	0.12	0.62	–0.13	0.04	–0.24
4	–0.25	0.11	0.87	–0.18	48
CV	10	11	14	20	29

(*c*) Estimated design-related dispersion matrix

Age					
0	1565	271	11.4	0.9	–69
1	0.56	149	60.5	1.9	10.5
2	0.07	*	17.4	3.1	54
3	*	*	*	(–4.8*)	1.1
4	–0.29	0.15	*	*	35
CV	9	10	9	*	25

*Invalid estimate (see text).

up keys for each station separately and for the North Sea as a whole. Station-based keys make no separation between length and age samples and so are unaffected by spatial (or temporal) variability of length-at-age across the region. Regional keys, on the other hand, appear more precise because of the aggregation of otoliths from several catches, but may yield biased estimates of numbers-at-age if length-at-age varies across the region. When comparing the estimates of numbers-at-age computed in the example, it can be seen that the relatively low precision of the station-based keys used for the estimates in Table 7*a* was smoothed out by the averaging of estimated numbers-at-age by station over the region, and the final estimates were comparable with those in Table 6 based on the regional key. Station-based keys require fewer estimation equations than regional keys, suggesting that they would be easier to comprehend and analyse statistically and that strategies for optimising otolith sampling would be easier to devise. In addition, estimates of numbers-at-age by station offer finer resolution for spatial or environmental studies than estimates by region. These arguments call into question the value of regional keys for groundfish surveys.

Weighing variances were small in the example because of the high quality of the balances used on the EGFS. Surveys not so well equipped, or that rely on raising by volume rather than weight, could be more severely affected. An unexpected effect of including weighing variance in the example was the appreciable increase in the variance of numbers of 0-year-olds and the almost halved magnitude of negative correlation between 0- and 1-year-olds (Table 6). Reasons can be found in eq. 15. Inclusion of weighing variance causes $\text{cov}(\hat{N}_l, \hat{N}_{l'})$ to increase for any pair of l and l', and since the P terms in the double summation are 1 for $a,a' = 0$ at most lengths (Table 4), these increases are almost completely incorporated in the total, leading to the large change in the variance for numbers of 0-year-old fish. The correlation between 0- and 1-year-olds was affected mainly by the changed denominator. In practical terms, these effects of weighing variance were only revealed by a change of CV for 0-year-olds from 1 to 2%, so that for surveys equipped with good balances, the complications of weighing variance could safely be ignored and eq. 9*b* used in place of eq. 10.

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Annex: Covariance of products

Bohrnstedt and Goldberger (1969) gave a general formulation for the covariance of products, but without describing estimators for the specific case needed here. For random variables x_1, x_2, y_1, and y_2, both x independent of both y, an estimator for the covariance of products xy can be found as follows. Following Goodman (1960), denote $E[x] = X$, and similarly for Y. Then, for $i = 1, 2$

$$x_i y_i - X_i Y_i = X_i Y_i \, (\delta x_i + \delta y_i + \delta x_i \delta y_i)$$

where $\delta x_i = (x_i - X_i)/X_i$ and $\delta y_i = (y_i - Y_i)/Y_i$. Therefore

$$\begin{aligned}\text{Cov}(x_1y_1, x_2y_2) &= X_1 Y_1 X_2 Y_2 \\ &\quad \times E[(\delta x_1 + \delta y_1 + \delta x_1 \delta y_1)(\delta x_2 + \delta y_2 + \delta x_2 \delta y_2)] \\ &= X_1 X_2 \cdot \text{Cov}(y_1, y_2) + Y_1 Y_2 \cdot \text{Cov}(x_1, x_2) \\ &\quad + \text{Cov}(x_1, x_2) \cdot \text{Cov}(y_1, y_2).\end{aligned}$$

Substituting the unbiased estimators $[x_1x_2 - \text{cov}(x_1, x_2)]$ for X_1X_2 and $\text{cov}(x_1, x_2)$ for $\text{Cov}(x_1, x_2)$, and similarly for Y variables, leads to the unbiased covariance estimator

$$\begin{aligned}\text{cov}(x_1y_1, x_2y_2) &= x_1x_2 \cdot \text{cov}(y_1, y_2) + y_1y_2 \cdot \text{cov}(x_1, x_2) \\ &\quad - \text{cov}(x_1, x_2) \cdot \text{cov}(y_1, y_2).\end{aligned}$$

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A process for developing and evaluating indices of fish assemblage integrity

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Abstract: We describe a general process for developing an index of fish assemblage integrity, using the Willamette Valley of Oregon, U.S.A., as an example. Such an index is useful for assessing the effects of humans on entire fish assemblages, and the general process can be applied to any biological assemblage and any region. First, a reference condition was determined from historical information, and then candidate metrics of ecological importance were listed. The variability of the candidate metrics in time and space was estimated and their responsiveness to independent measures of riparian and stream habitat quality assessed. Metrics were scored continuously from 0 to 10, producing an index of biological integrity (IBI) that was weighted to range from 0 to 100 regardless of the number of metrics. The index, developed from a set of 35 sites, was then tested on an independent set of eight urban sites sampled by the Oregon Department of Fish and Wildlife. Thirteen of the 16 candidate metrics were appropriate and produced an IBI with among-site variance triple that of revisit variance. The method distinguished sites with acceptable fish assemblages from marginally and severely impaired sites.

Résumé : Nous décrivons un processus général pour l'élaboration d'un indice de l'intégrité des groupements de poissons, en utilisant la vallée de la Willamette, en Oregon, aux États-Unis, à titre d'exemple. Un indice de cette sorte est utile pour évaluer les effets des activités humaines sur des groupements entiers de poissons, et le processus général peut être appliqué à tout groupement biologique et dans n'importe quelle région. En premier lieu, nous avons déterminé un état de référence à partir d'informations historiques, puis nous avons dressé une liste de paramètres pertinents sur le plan écologique. Nous avons estimé la variabilité des paramètres dans le temps et dans l'espace et évalué leur réactivité à des mesures indépendantes de la qualité de l'habitat riverain et fluvial. Les paramètres étaient évalués sur une échelle continue allant de 0 à 10 pour produire un indice de l'intégrité biologique (IIB) qui a été pondéré de façon à s'étendre de 0 à 100, quel que soit le nombre de paramètres. L'indice, établi pour un ensemble de 35 sites, a ensuite été testé sur un ensemble indépendant de huit sites urbains échantillonnés par l'Oregon Department of Fish and Wildlife. Treize des 16 paramètres considérés étaient appropriés et ont produit un IIB ayant une variance inter-sites trois fois supérieure à la variance recalculée. Cette méthode a permis de repérer des sites abritant des groupements de poissons acceptables par rapport aux sites peu ou très dégradés.

[Traduit par la Rédaction]

Introduction

Biological integrity, an objective of the 1972 U.S. Clean Water Act and Canada's National Park Act, has been defined as the ability to support and maintain a balanced, integrated, adaptive community of organisms having a composition, diversity, and functional organization comparable with that of the natural habitats of the region (Frey 1977). We consider a community as having integrity if its composition and function are comparable with those of natural habitats, which we define as minimal human disturbance, or pre-Columbian conditions. Such conditions are only benchmarks for comparison and may not be achievable given current land uses.

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Biological integrity is best monitored at the community or assemblage level because indicator species or populations are insufficiently robust indicators (Landres 1992). We agree with Fauth et al. (1996) and use the term "assemblage" to represent a phylogenetic subset of a community, while a community is the entire biological component of an ecosystem. A widely used indicator of fish assemblage integrity is the index of biological integrity (IBI, Karr et al. 1986). Initially developed for midwestern U.S. streams, it has been modified for streams of Appalachia, Ontario, North Carolina, Colorado, Tennessee, Idaho, Missouri, and Mexico, large rivers in Oregon, France, Ohio, Australia, Africa, Belgium, and India, Tennessee river reservoirs, and Great Lakes bays (Simon and Lyons 1995; Hughes and Oberdorff 1998). However, few applications included evaluations of metric and IBI variability and responsiveness to disturbance; Minns et al. (1994) is a notable exception.

IBIs reflect important components of ecology: taxonomic richness, habitat and trophic guild composition, and individual health and abundance. Metrics in each category are scored and then summed into an IBI value. Differences in expected fish species richness and composition associated with different regions or basins, water body sizes, and location in a drainage are factored into metric selection and scoring (Fausch et al. 1984; Miller et al. 1988). IBIs focus on fish assemblage

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structure rather than ecosystem processes because the latter appear less responsive to stressors (Schindler 1990). As recommended by Karr et al. (1986), however, both structural and functional metrics are included. IBIs translate an aquatic ecologist's assessment of fish assemblage integrity for persons unfamiliar with fishes. They alone cannot convey causal relationships or fundamental ecological processes.

Our objectives were to demonstrate a general method for developing and evaluating biological indicators of biological integrity that can be useful for monitoring across large regions for long time periods. As an example, we describe how we determined reference conditions, developed an IBI for Willamette Valley, Oregon, U.S.A., fish assemblages, assessed the variance of IBI metrics, compared IBI scores with physical and chemical habitat, and tested the IBI on an independent data set.

Methods

Reference condition

Commonly, natural condition is estimated from minimally disturbed sites, but in heavily altered regions, such sites are absent. In those cases, historical data, paleoecological data, quantitative models, and expert judgement are used to define the natural condition (Hughes 1995). Willamette Valley streams are widely disturbed by agriculture and urbanization. Therefore, we based the reference condition on estimates of pre-Columbian hydrological and stream habitat characteristics, fish–habitat relationships, and the regional fish species pool as proposed in Hughes (1995). These were developed from an evaluation of historical museum data (Hughes et al. 1987), nine summers of stream sampling throughout the Valley, conversations about past stream and fish assemblage conditions with emeritus professors of ichthyology (R. Dimick and C. Bond, Oregon State University, Corvallis, OR), and reviews of historical conditions (Johannessen et al. 1971; Benner and Sedell 1997). We developed candidate IBI metrics based on our interpretation of historical conditions and the habitat and trophic requirements of the Valley's fish species (Table 1). Fausch et al. (1984) used a similar approach for species richness and guild types of metrics across a greater range of stream sizes.

Candidate metrics

Following the lead of Karr et al. (1986), we identified candidate metrics that represent major aspects of fish assemblage integrity: taxonomic richness, habitat guilds, trophic guilds, and individual health and abundance. To increase resolution among sites and reduce variance, we also modified their scoring criteria (discussed below).

Taxonomic richness

Karr et al. (1986) focused on species richness. We substituted native species richness as they recommended for streams of the western United States. We did not want to confound the species richness metric, which frequently decreases with degradation, with alien species, which represent biological degradation and homogenization and often increase with disturbance. We also added a metric, native family richness, to assess an additional level of biological diversity. Oberdorff and Hughes (1992) reported that entire families were extirpated from, or threatened in, basins with long and intensive human occupation.

Habitat guilds

Karr et al. (1986) used the number of darter, sunfish, sucker, and intolerant species to evaluate fish assemblage components likely to decrease in response to habitat degradation. Because darters and sunfish are alien to Oregon, we followed the suggestion of Karr et al. (1986) and Miller et al. (1988) and used native species richness of benthic and water column fishes instead. Habitats for each species (Table 1) were determined from Scott and Crossman (1973), Moyle (1976), Becker (1983), and Bond et al. (1998). Suckers only rarely occurred in Willamette Valley streams; a sucker metric would therefore be insensitive, so we did not include one. In place of intolerant species richness, we used sensitive species richness and the species richness of nonguarding lithophil nesters. These species are early-warning indicators of anthropogenic disturbance and they rarely occur in highly turbid, warm, chemically polluted, or heavily silted streams. They occur most often in clear, cool, unpolluted streams with complex cover and coarse substrates (although they tolerate extreme flows, cold temperatures, and oligotrophic conditions). Oberdorff and Hughes (1992) also used a lithophil metric. Because such species were rarely abundant in even the least disturbed Willamette Valley streams, we based these metrics on species richness rather than proportions of individuals of these species in the assemblage. We also evaluated native hider species richness. Hiders (Table 1) are fishes commonly found in substrate interstices or among macrophytes or organic debris. Because they were difficult to collect and rarely abundant, we again based the metric on the number of hider species rather than the proportion of individuals collected that are hiders.

To assess assemblage components that increase with habitat disturbance, we substituted percent alien (nonnative) and percent tolerant individuals in the sample for the percent green sunfish metric originally used by Karr et al. (1986). The percent aliens reflects biological pollution, which is usually less reversible than chemical and physical disturbance; it was also the second most commonly cited cause of fish species extinction after physical habitat change (Miller et al. 1989). Karr et al. (1986) and Miller et al. (1988) also suggested substituting percent tolerants for percent green sunfish. Tolerants (Table 1) were determined from Scott and Crossman (1973), Moyle (1976), and Becker (1983); such species are generally successful in streams with high silt loads and warm, turbid, and poorly oxygenated water. All tolerants in our streams were aliens, although not all the aliens were considered tolerant. Several commonly occurring native species were not included as tolerants because they are are less tolerant than the aliens and can be very abundant in minimally disturbed systems. We used percent tolerant individuals in the sample versus tolerant species richness, which helped us assess the tendency for only one of these species to dominate in highly disturbed situations or where few individuals persisted.

Trophic guilds

Karr et al. (1986) used percent individuals as omnivores, insectivores, and piscivores to evaluate the status of the trophic structure and food base. We adopted the omnivore metric, but rejected the other two. Omnivores typically eat substantial amounts of both plant and animal material, allowing such species to adapt easily to disrupted food sources. There are very few true piscivores in Willamette River basin streams and most individuals were invertivores. This meant that regardless of their relative integrity, streams lacked piscivores. Invertivores, on the other hand, were very abundant in all streams and did not demonstrate marked differences in proportions as habitat quality differed, which resulted in unresponsive metrics. We substituted percent filter feeders and percent native top carnivores for the invertivore and piscivore metrics. Filter feeders (juvenile lampreys) are sensitive to migratory barriers, suspended sediment concentration, and the quality of fine particulate organic matter and microorganisms in the drift and bedload. The top carnivore metric evaluates the ability of the stream to produce enough fish and large invertebrates to support relatively large native predators. It was suggested as a piscivore substitute by Karr et al. (1986). We used Scott and Crossman (1973), Moyle (1976), Wydoski and Whitney (1979), Becker (1983), and Li et al. (1987) to designate trophic guilds for each species (Table 1).

Individual health and abundance

The total number of individuals and percent individuals that are hybrids or diseased were used by Karr et al. (1986) to assess stream

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Table 1. Fishes of wadeable streams in the Willamette Valley ecoregion.

Family/species	Origin[a]	Habitat[b]	Tolerance[c]	Foraging[d]	Reproduction[e]
Petromyzontidae					
Lampetra richardsoni	N	BH	S	F/S	NLN
Lampetra tridentata	N	BH	S	F/S	NLN
Cyprinidae					
Acrocheilus alutaceus	N	B	I	S/S	L
Carassius auratus	A	B	T	O	V
Ctenopharyngodon idella	A	B	T	H	V
Cyprinus carpio	A	B	T	O	V
Mylocheilus caurinus	N	W	I	I	L
Oregonichthys crameri	N	WH	S	I	V
Ptychocheilus oregonensis	N	W	I	T	L
Pimephales promelas	A	B	T	O	P/CN
Rhinichthys cataractae	N	BH	I	I	L
Rhinichthys falcatus	N	BH	I	I	L
Rhinichthys osculus	N	BH	I	I	LN
Richardsonius balteatus	N	W	I	I	LV
Cobitidae					
Misgurnus anguillicaudatus	A	B	T	O	V
Catostomidae					
Catostomus macrocheilus	N	B	I	O	L
Catostomus platyrhynchus	N	B	S	S/S	L
Ictaluridae					
Ameiurus melas	A	BH	T	O	P/CN
Ameiurus natalis	A	BH	T	O	P/CN
Ameiurus nebulosus	A	BH	T	O	P/CN
Salmonidae					
Oncorhynchus clarki	N	WH	S	T	NLN
Oncorhynchus kisutch	N	W	S	T	NLN
Oncorhynchus mykiss	N	WH	S	T	NLN
Oncorhynchus tshawytscha	N	W	S	T	NLN
Prosopium williamsoni	N	B	S	I	NLN
Percopsidae					
Percopsis transmontana	N	BH	I	I	L
Fundulidae					
Fundulus diaphanus	A	W	T	O	V
Poeciliidae					
Gambusia affinis	A	WH	T	O	LB
Gasterosteidae					
Gasterosteus aculeatus	N	WH	I	I	VN
Cottidae					
Cottus asper	N	B	I	I	CN
Cottus beldingi	N	BH	S	I	CN
Cottus gulosus	N	BH	I	I	CN
Cottus perplexus	N	BH	I	I	CN
Cottus rhotheus	N	BH	S	T	CN
Centrarchidae					
Lepomis auritus	A	W	T	I	PN
Lepomis cyanellus	A	W	T	T	PN
Lepomis gibbosus	A	W	I	I	PN
Lepomis gulosus	A	W	T	T	PN
Lepomis macrochirus	A	W	T	I	PN
Lepomis microlophus	A	W	T	I	PN
Micropterus dolomieu	A	W	I	T	LN
Micropterus salmoides	A	W	T	T	PN
Pomoxis annularis	A	W	T	T	VN

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Table 1 (*concluded*).

Family/species	Origin[a]	Habitat[b]	Tolerance[c]	Foraging[d]	Reproduction[e]
Pomoxis nigromaculatus	A	W	T	T	PN
Percidae					
Perca flavescens	A	W	I	T	V

Note: Data from Scott and Crossman (1973), Moyle (1976), Wydoski and Whitney (1979), Becker (1983), Li et al. (1987), and Bond et al. (1988).

[a]N, native; A, alien.

[b]B, benthic; W, water column; H, hider.

[c]S, sensitive; I, intermediate; T, tolerant.

[d]F/S, filterer/specialist; S/S, scraper/specialist; O, omnivore; I, invertivore; T, top carnivore.

[e]NLN, nonguarding lithophil (gravel–cobble) nester; LN, lithophil nester; L, lithophil; V, vegetation; P, psammophil (sand – fine gravel); CN, cavity nester; LB, livebearer; VN, vegetation nester; PN, psammophil nester.

productivity and individual fitness. The hybrid metric was excluded because hybrids were rare, or we lacked the ability to recognize them. We included a metric for percent individuals that were diseased or had external anomalies. Typically, such an anomaly metric is most useful with larger fish and in waters that are severely degraded by chemical contaminants. We also examined a "total number of individuals" metric, although both oligotrophic and highly disturbed physical or chemical habitats typically support exceedingly low fish numbers. To evaluate the effects of degradation on long-lived individuals, we examined the percent individuals that were native adults and the percent long-lived native species with individuals reaching large sizes ("lunkers," Table 2). These two metrics also assess flow permanence and habitat suitability as well as the site's ability to support adults. Johnson (1994) reported that minimally disturbed waters were frequently dominated by large, old individuals.

Metric and index scoring

Karr et al. (1986) scored metrics as 5, 3, or 1 if their data approximated, deviated slightly from, or were markedly different from reference conditions. Thus the 12 metrics they used produced an index ranging from 12 to 60 if fish were present. We agree with Minns et al. (1994) that an index based on continuous scoring of 0.0–10.0 for metrics and 0.0–100.0 for the IBI is preferable. The decimal system is a familiar one, as are ranges from 0 to 10 or 100. Also, continuous scoring, including decimal fractions, reduces variances when metric values differing by a value ≤ 1 are scored as different categories. These changes, by offering more accurate depiction of the data, should make IBIs less variable and more easily understood.

We set the upper and lower thresholds for each metric based on our interpretation of pre-Columbian stream conditions, species ranges, fish habitat requirements, and reference sites (Table 2). Upper thresholds (scores of 10) vary with stream size and among metrics. Lower thresholds (scores of 0) were 0 for all metrics except percent alien, percent tolerant, percent omnivores, and percent anomalies; these were set at 10, 10, 10, and 2, respectively. These latter four metrics received higher metric scores as data values decreased to 0. Scores between the upper and lower thresholds were calculated by linear interpolation (dividing the metric value by its range and then multiplying by 10) as described by Minns et al. (1994). For example, the maximum expected value for the hider metric was four species, so three hider species received a score of 7.5. IBI scores are the sums of the metric scores times 10 and divided by the number of metrics, producing a maximum IBI score of 100 regardless of the number of metrics selected in future applications. Minns et al. (1994) also used this approach.

Table 2. Scoring criteria for IBI metrics used in order 1–3 streams.

	Raw values	
Metric	Stream order 1	Stream orders 2 and 3
Taxonomic richness		
No. of native families	0–4	0–7
No. of native species	0–5	0–11
Habitat guilds		
No. of native benthic species	0–3	0–7
No. of native water column species	0–2	0–4
No. of hider species	0–4	0–4
No. of sensitive species	0–2	0–5
No. of native nonguarding lithophil nester species	0–3	0–3
% tolerant individuals	10–0	10–0
% alien individuals[a]	10–0	10–0
Trophic guilds		
% filter-feeding individuals	0–10	0–10
% native top carnivore individuals	0–10	0–10
% omnivores	10–0	10–0
Individual health and abundance		
% of target species that include lunkers[b]	0–100	0–100
% individuals with anomalies	2–0	2–0
% native species with adult individuals[a]	0–100	0–100
Total no. of individuals[a]	0–50	0–50

Note: Raw data values at the low (and lower) end of the ranges are scored as 0; those at the high (and higher) end are scored as 10; scores for intermediate values are calculated by dividing the raw value by the score range (see text for examples). Strahler stream orders determined from 1 : 100 000 scale maps.

[a]Metric not included in final IBI.

[b]Lunkers are relatively old large individuals of the following species and sizes: *C. asper* (10 cm), *C. rhotheus* (10 cm), *O. clarki* (25 cm), *O. mykiss* (30 cm), *A. alutaceus* (30 cm), *P. oregonensis* (30 cm), *C. macrocheilus* (30 cm). See Table 1 for full species names.

Data sources

Our data originated from a set of 21 subjectively chosen stream sites sampled three times in the summer of 1982 and 18 randomly selected stream sites sampled in 1993. Eight of the latter 18 streams were also sampled twice in the summers of 1992–1995 to assess variance components (Herlihy et al. 1997). All streams were in the Willamette River basin and the Willamette Valley ecoregion (Omernik 1987). Data from randomly selected reaches ensured that data were representative of most Valley streams, while data from the subjectively chosen sites ensured that extremes in stream quality were represented. Stream reaches were sampled for fish with backpack electrofishers during the summer base flow period, but effort varied

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from three passes on 100 m in 1982 to one pass on reaches 40 times as long as their wetted channel widths in 1992–1995 (McCormick and Hughes 1997). A separate set of eight subjectively chosen sites electrofished by three passes of 100 m in 1993 and 1994 (Friesen and Ward 1996) was used as an independent test of the IBI.

Water quality samples and physical habitat data were taken during each visit to the randomly selected reaches (Herlihy et al. 1997; Kaufmann and Robison 1997). Physical habitat was sampled quantitatively by two persons for 3–4 hours. It included a count of the large woody debris and 100 measurements of depth along the thalweg. In addition, at 11 cross sections, measurements of width, bank height and angle, gradient, sinuosity, and riparian canopy cover were taken, and substrate size, embeddedness, riparian vegetation condition, fish cover, and human disturbances were visually estimated. A 4-L cubitainer of stream water was collected in a flowing portion near the middle of the stream for major anion, cation, conductivity, and nutrient analyses. Detailed information on the analytical and preservation procedures for each analyte can be found in USEPA (1987). In brief, major anions (sulfate, nitrate, chloride) were determined by ion chromatography, base cations by atomic absorption, and total nitrogen and phosphorus by persulfate oxidation and colorimetry. Dissolved oxygen (DO) concentrations for the Tualatin River basin (an urbanized subbasin of the Willamette) sites were provided by the Unified Sewerage Agency. Those data were collected weekly in the summers of 1990–1994 between 08:00 and 09:50 through use of daily calibrated DO meters.

Data analyses

We determined if the metrics were sensitive at high, low, and intermediate levels of biological integrity, a desirable trait for an accurate and useful IBI. Initially, we sorted IBI and metric scores to help discern ranges and patterns; this led to modifications of scoring criteria by stream order for five metrics. Next, we plotted metric scores and raw metric values against IBI.

To eliminate metrics that were redundant and those that consistently masked the signal of other metrics, we calculated correlation coefficients for index and metric scores. We anticipated high positive correlations between the IBI and individual metrics and among the metrics, yet no identical correlations between metrics and the IBI. The sorted IBI and metric scores and the correlations aided us in finding metrics that behaved in a different manner than the others (consistent negative correlations). Cronbach's alpha (Cronbach 1951) and a metric-remainder correlation coefficient were also used. The metric-remainder coefficient was determined by calculating the IBI without the metric and then calculating the correlation between this modified IBI and the metric value. A metric that was concordant with the others had a large, i.e., close to 1, positive coefficient. Cronbach's alpha was determined by dividing the sum of all pairwise metric covariances by the total IBI variance, which included both metric variances and covariances. For a metric consistent with the others, its alpha will decrease relative to the alpha value for the IBI. Conversely, for a metric inconsistent with the others, its alpha will increase relative to the IBI alpha value. Cronbach (1951) recommended that alpha should be around 0.7 for a useful metric or index. This value signals a suitable level of redundancy, yet reduces opportunities for some metrics to consistently counter the scores of other metrics.

IBI and metric variability were evaluated in three ways. First, we plotted IBI and metric scores obtained from multiple samplings of the same sites against each other in 1:1 plots. The precision of the IBI in such plots was assessed by the proximity of the points to a 45° line. These plots also offered a simple visual means to determine if variability was greater for high or low IBI scores. Second, analysis of variance was used to compare the relative contributions of major sources of variance (Larsen et al. 1995). These included among-site (the variance resulting from differences among all sites sampled), year-to-year (the degree that all sites trend in the same direction in a particular year), individual site–year interaction (the amount a site varied from year to year around its mean or trend), and residual or error variance (variance contributed by measurement methods, crew-to-crew differences, and temporal variance during the sampling season). We also determined the ratio of among-site variance to the sum of all other sources of variance for each metric and the IBI. Third, we determined power curves for three significance levels at various power values and differences in IBI scores. Power, the ability of the IBI to detect differences among samples, was evaluated through use of the variance components determined above. In particular, we wanted to know for a power of 0.8 and an α of 0.05 (*i*) the detectable difference in mean IBI scores from one year to the next and (*ii*) the number of years of data needed to detect a 2% per year trend in mean IBI score.

We tested IBI responsiveness with a set of quantitative physical and chemical habitat data from the 18 randomly selected sites. We applied principal components analysis (PCA) to 16 chemical and physical habitat variables to express an integrated measure of habitat quality against which we evaluated the candidate fish metrics and IBI.

We also applied PCA to the fish data to evaluate a multivariate measure of fish assemblage integrity. We used PCA of raw metric values and scored metrics and plotted the first two components to determine if PCA could discriminate different degrees of fish assemblage integrity. In other words, were sites with markedly different habitat quality located apart on the graph and separated by those of intermediate quality?

The proportion of the sampled sites that had acceptable, marginally impaired, or severely impaired fish assemblage integrity was estimated by tabulating IBI scores, or mean IBI scores from reaches with multiple samples. The scores were then compared with the maximum potential IBI score (100). Scores were considered acceptable, marginally impaired, or severely impaired if they were ≥ 75, 51–74, or ≤ 50, respectively. The upper scoring break was comparable with Karr et al.'s (1986) break between good and fair categories, while the lower break was close to Karr et al.'s 57% cutoff for poor sites. Scores were also evaluated by comparison with the maximum observed mean IBI score (90) at a reach. In this case, acceptable, marginally impaired, or severely impaired scores were ≥ 68, 46–67, or ≤ 45, respectively.

Results

Metric performance

Most scored metrics (versus raw metric values) were positively and highly correlated ($r > 0.5$) with IBI, and most correlations among metrics were positive. Total number of individuals, percent large fish, percent anomalies, and percent native adults had low correlations with IBI and all other metrics. All seven richness metrics were highly correlated with each other, even benthic and water column species richness. The percent alien and percent tolerant metrics were redundant in this data set ($r = 1$). Similarly, most metric-remainder correlation coefficients were high for the total data set, except for total number of individuals, percent anomalies, and percent native adults (all with $r \leq 0.3$, second to last column of Table 3). Cronbach's alpha scores for the total data set were all positive and = 0.7 if rounded (last column of Table 3). These results indicate a relatively high degree of concordance among metrics, especially for the longer reaches sampled in 1992–1995.

A multivariate (PCA) representation of physical and chemical habitat quality was calculated independently through use of quantitative data from the 1992–1995 sites (Table 4). Low values of habitat PCA-1 (HPCA-1) were associated with high levels of human disturbance at the site, as well as high chloride and sulphate concentrations. High HPCA-1 scores occurred

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Table 3. Values of the metric-remainder correlation coefficient for each scored metric and Cronbach's alpha for the IBI and for the restricted IBI associated with each of the scored metrics.

	1982, stream orders 2 and 3		1992–1995				1982–1995, all three data sets	
			Stream order 1		Stream orders 2 and 3			
	Coeff.	Alpha	Coeff.	Alpha	Coeff.	Alpha	Coeff.	Alpha
IBI		0.33		0.73		0.66		0.65
Native family richness	0.40	0.40	0.98	0.73	0.87	0.67	0.81	0.67
Native species richness	0.53	0.39	0.93	0.73	0.90	0.67	0.86	0.66
Hider species richness	0.72	0.33	0.91	0.74	0.49	0.69	0.76	0.66
Benthic species richness	0.44	0.40	0.81	0.74	0.84	0.67	0.79	0.67
Water column species richness	0.41	0.39	0.80	0.76	0.34	0.70	0.65	0.67
Sensitive species richness	0.82	0.34	0.83	0.74	0.70	0.68	0.84	0.66
Nonguarding lithophil nester richness	0.75	0.32	0.97	0.73	0.73	0.67	0.85	0.65
Total no. of individuals	0.06	0.44	0.68	0.75	0.47	0.69	0.24	0.70
% filter-feeding individuals	0.54	0.36	0.68	0.75	0.74	0.67	0.68	0.67
% alien individuals	0.62	0.33	0.35	0.77	0.68	0.68	0.54	0.68
% native top carnivore individuals	0.47	0.37	0.78	0.74	0.53	0.69	0.60	0.68
% tolerant individuals	0.62	0.33	0.31	0.77	0.69	0.68	0.55	0.68
% target species with lunkers	0.21	0.41	0.25	0.77	0.48	0.69	0.35	0.69
% individuals with anomalies	–0.01	0.49	—[a]	0.77	—[a]	0.71	0.20	0.71
% omnivorous individuals	0.13	0.47	0.31	0.77	0.54	0.69	0.37	0.70
% native adult individuals	0.01	0.43	0.45	0.76	0.38	0.70	0.29	0.70

[a]Values of the metric were constant for this data set.

where riparian vegetation was abundant and multilayered, instream fish cover was common and diverse, and residual pool volume was high.

Raw metric data from the 1993 samples demonstrated expected, but frequently weak and variable, patterns when plotted against HPCA-1 scores. The limited number of points often did not indicate strong associations between metric data and HPCA scores (Fig. 1). Three metrics (percent alien, percent tolerant, percent omnivores) showed high values only at sites with low habitat quality. A set of four metrics (native nonguarding lithophil nesters, percent filter feeders, percent native top carnivores, percent species with large individuals) showed the opposite pattern: high values only occurred when habitat quality was high. Data for percent native adults appeared unrelated to habitat quality. The remaining metrics increased constantly with increased habitat quality. The candidate metrics, therefore, included some that appeared sensitive at either high or low extremes of habitat quality as well as those responsive across the range of habitat quality expected in most Willamette Valley streams. When all the metrics were scored, combined into an IBI, and plotted against habitat quality, there was a significant ($r = 0.65$, $p < 0.01$) positive relationship.

Because of small sample size and considerable variability, Fig. 1 provided weak evidence for metric responses to habitat quality. To the degree that IBI is also a measure of ecological integrity and habitat quality (as suggested by the highly significant relationship between the two), we can examine patterns of raw metric scores plotted against IBI scores for the combined 1982 and 1992–1995 data (Fig. 2). All but the total number of individuals and the percent native adult metrics showed the expected patterns.

As a result of these analyses, three metrics (total number of individuals, percent native adults, percent alien) were removed from the final IBI. Total number of individuals and percent native adults were unresponsive to general habitat disturbance and the IBI score, while percent alien and percent tolerant were identical. A fourth metric, percent anomalies, was retained because of insufficient data for rejection.

Table 4. Eigenvectors and accountable variances of the first two principal components based on physical and chemical habitat variables.

PCA-1	PCA-2	Variable
0.84	0.32	Mean woody cover (ground, midlayer, canopy)
0.82	0.28	Mean canopy cover in riparian zone
0.79	0.09	Mean large woody debris in flooded channel
0.76	0.22	Mean small woody debris in flooded channel
0.71	–0.05	Mean width × depth
–0.66	0.01	Anthropogenic disturbances within 10 m of stream
0.58	–0.08	Residual pool area
0.53	–0.17	Standard deviation of width
–0.63	0.43	Sulphate
–0.58	0.46	Conductivity
–0.58	0.34	Chloride
–0.31	0.66	Total nitrogen
–0.21	0.36	Mean vegetation cover overhanging stream
0.25	0.86	% fines and sand
–0.28	–0.83	Mean substrate size
37	18	% variance accounted for

PCA of IBI metrics

PCA of species abundance data did not produce interpretable patterns, but a PCA using IBI metrics (BPCA) did. The first and second principal component factors of metric scores accounted for 44 and 13% of metric score variability, respectively (Table 5). All 13 metrics were positively associated with BPCA-1, which further indicated internal consistency among the IBI metrics. The richness metrics loaded more highly

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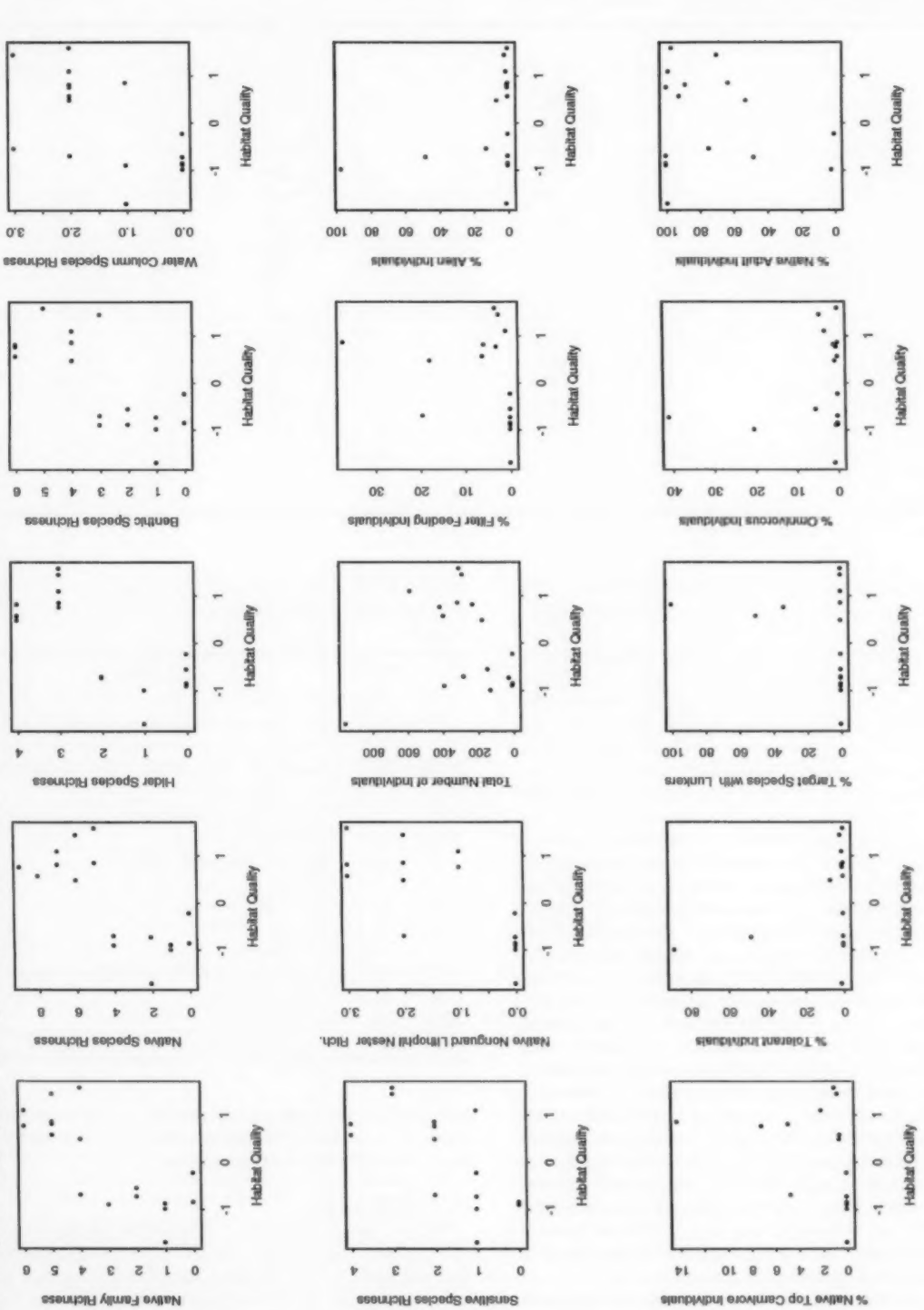

Fig. 1. Raw metric scores versus physical and chemical habitat quality for 15 metrics. Habitat quality was estimated from PCA-1 (see Table 4) and increases from left to right. Metrics showed constantly increasing, rapidly decreasing, slowly increasing, or highly variable responses to increasing habitat quality.

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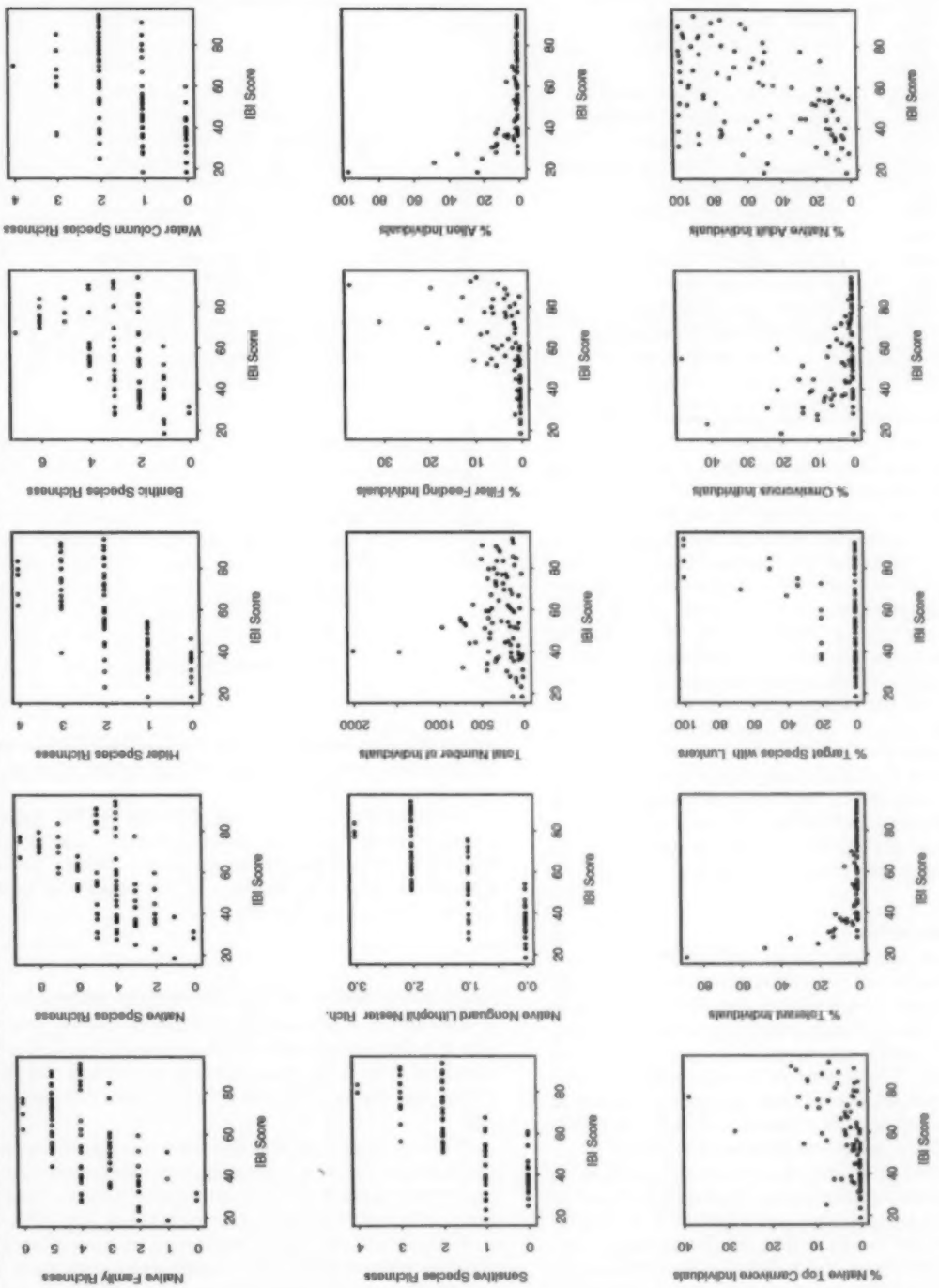

Fig. 2. Raw metric scores versus IBI scores. Metrics showed constantly increasing, rapidly decreasing, slowly increasing, or highly variable responses to increasing IBI scores.

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Table 5. Eigenvectors and accountable variances of the first two principal components based on IBI metric scores.

PCA-1	PCA-2	IBI candidate metric
0.34	–0.18	Native fish family richness
0.35	–0.15	Native fish species richness
0.32	–0.16	Hider species richness
0.32	–0.06	Native benthic species richness
0.28	–0.18	Water column species richness
0.33	–0.04	Sensitive species richness
0.34	–0.05	Nonguarding lithophil nester richness
0.13	–0.27	Total no. of individuals
0.23	0.04	% filter-feeder individuals
0.19	0.52	% alien individuals
0.21	–0.06	% top carnivore individuals
0.19	0.52	% tolerant individuals
0.16	0.03	% target species with lunkers
0.09	–0.05	% individuals with anomalies
0.15	0.49	% omnivorous individuals
0.15	0.17	% native adult individuals
44	13	% variance accounted for

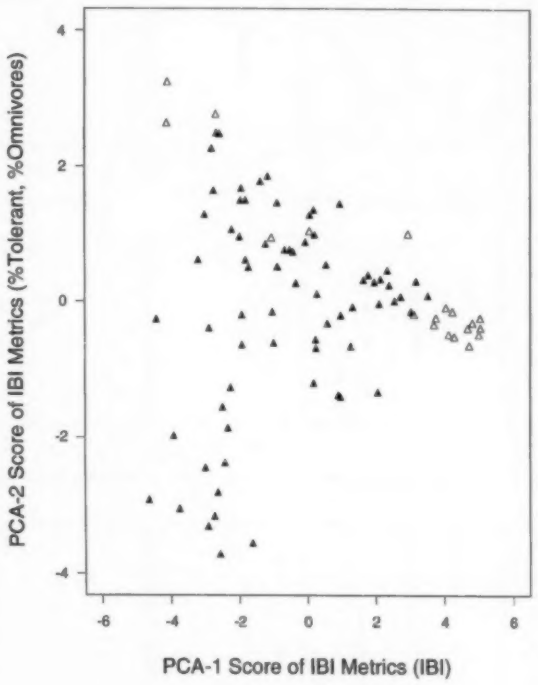
(0.28–0.35) than the percent metrics (0.09–0.23). These results suggest that BPCA-1 may be considered an IBI. This was also indicated by the close association of IBI and BPCA-1 scores ($r = 0.99$). BPCA-2 correlated most with the IBI metrics indicating disturbance (percent tolerant, percent alien, percent omnivores). When BPCA-1 and BPCA-2 were plotted together, sites with low scores on both axes (lower left of Fig. 3) had few omnivorous or tolerant (alien) species, yet had low IBI scores. There was also a negative relationship between BPCA-1 and BPCA-2 for small (first-order) streams, indicating that omnivorous or tolerant (alien) species declined as IBI increased in these streams. PCA of raw metric values (not shown) gave similar results. These findings indicated that IBI metrics ranked fish assemblage integrity whether aggregated through use of a multimetric index or a multivariate analysis. Successful extraction of this pattern, however, was based on analysis of a set of ecologically meaningful metrics, not the typical ordination of species and abundances.

IBI variance and power

Variances were estimated for the 1992–1995 data, which included both interannual and intraseasonal repeat sampling. Among-site variance comprised the preponderance of total variance in IBI, with considerable stream/year interaction variance, and much smaller among-year variance and residual variance (Table 6). This is also true for most individual metrics, except for percent filter feeders, percent top carnivores, percent lunkers, and percent omnivores. Fore et al. (1994) found similar patterns in sources of variance for IBI.

A simple graphic of variance was obtained by plotting the IBI results of repeat sampling against the mean IBI score of multiple samples collected at the site in 1982 (Fig. 4). Similar plots were examined for seven sites sampled multiple years (not shown). In both cases, and as indicated by Table 6, temporal differences in IBI scores were typically less than 10% (10 units) of the potential IBI maximum and substantially lower than differences among sites. When the standard deviations of IBI scores were plotted against mean IBI scores for sites with repeat samples, low IBI scores reflected lower standard deviations than intermediate or high scores, although the pattern was weak (Fig. 5).

Fig. 3. First and second principal component scores from IBI metrics (open triangles, first-order streams; solid triangles, second- and third-order streams). Stream sites in the lower left had few omnivorous and tolerant (alien) individuals but low IBI scores.



Statistical power analyses indicated that our IBI, based on a sample size of 18 streams each sampled once, can detect an 8-unit (8%) year-to-year difference in mean IBI at a power of 0.8 and $\alpha = 0.05$ (Fig. 6A). A 2-unit (2%) per year trend is detectable in 5 years (Fig. 6B).

Independent validity test and impairment assessment

Another test of the usefulness of an IBI is its performance with an independently collected and assessed data set. Our IBI, calculated from 1994 data provided by Friesen and Ward (1996), was highly correlated ($r = 0.85$) with DO concentration.

The proportion of all sites that were in acceptable, marginally impaired, or severely impaired condition depended on the evaluation criteria. Depending on whether the maximum potential IBI score (100) or the maximum actual score (90) was used, 84–91% of sites were considered marginally or severely impaired, while 9–16% were considered in acceptable condition. These percentages reflected the biological criteria and the site selection process (both subjectively chosen and randomly selected), as well as the extent of landscape disturbance in the Willamette Valley.

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Table 6. IBI and individual metric variances for 1992–1995.

	Among-site	Year	Stream × year	Residual	Ratio[a]
Family richness	6.15	–0.03	0.72	1.07	3.44
Species richness	5.27	0.12	1.15	0.84	2.50
Hider richness	9.52	0.09	0.39	1.94	3.93
Benthic richness	8.13	0.64	0.59	0.62	4.39
Water column richness	5.33	0.63	2.20	2.50	1.00
Sensitive species richness	9.62	0.03	5.21	0.70	1.62
Nonguarding lithophil nester richness	12.36	–0.53	1.91	2.78	2.64
% filter-feeding individuals	3.28	–0.81	3.59	5.73	0.35
% top carnivore individuals	3.26	3.66	7.55	0.66	0.27
% tolerant individuals	8.29	0.32	2.32	0.99	2.28
% target species with lunkers	2.84	0.00	–4.48	11.53	0.25
% individuals with anomalies	—	—	—	—	—
% omnivorous individuals	4.55	0.26	3.46	1.27	0.91
IBI	374.87	–16.89	98.65	26.13	3.00

[a]Among-site variance divided by sum of all remaining positive terms. By definition, variance components are positive, but negative variances can occur when variance is close to zero. We view the negative estimate as evidence that the true value of that component approximates zero.

Fig. 4. IBI precision as indicated by 1 to 3 monthly sampling visits at the same site in a summer. Numbers represent months sampled (1 = June, 2 = July, 3 = August), and their vertical positions indicate the IBI score on that visit versus the mean of all visits to that site. Mean IBI scores among sites ranged from 25 to 71, while the ranges of IBI scores at a site were usually <10. Also, low mean IBI scores (<40) varied less from the 1:1 diagonal line than did intermediate and higher values.

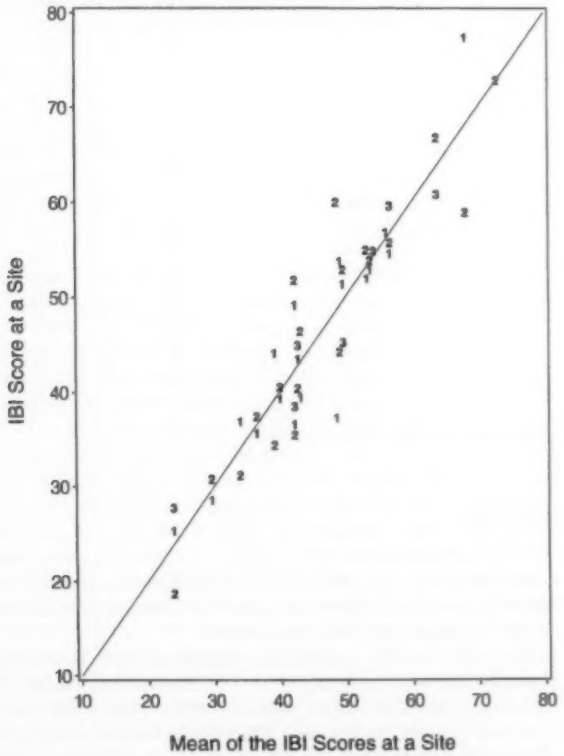

Fig. 5. IBI standard deviation as a function of mean IBI scores at sites sampled multiple times (8 = 1982 sites, 9 = 1992–1995 sites). Lowest mean IBI scores (<40) did not have high standard deviations.

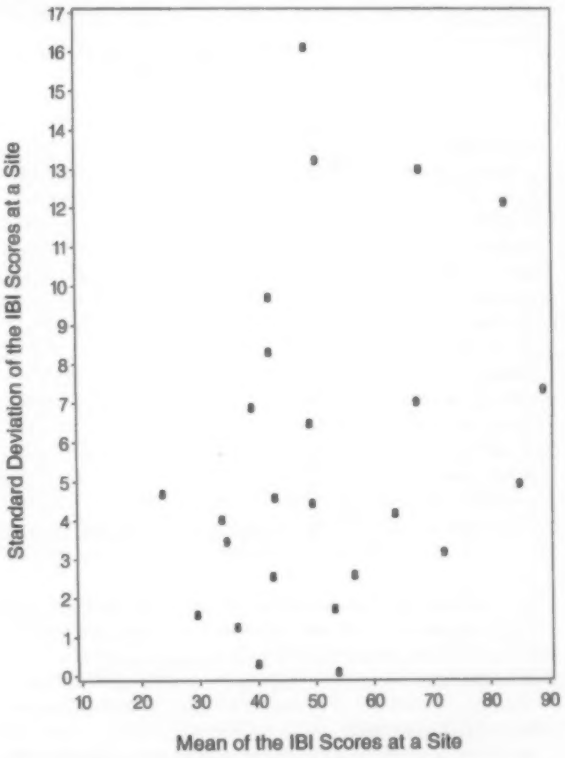

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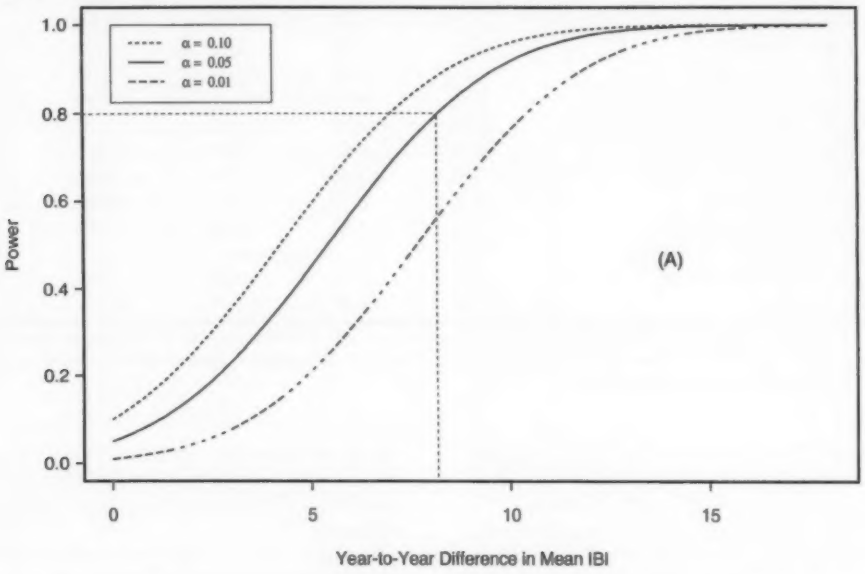
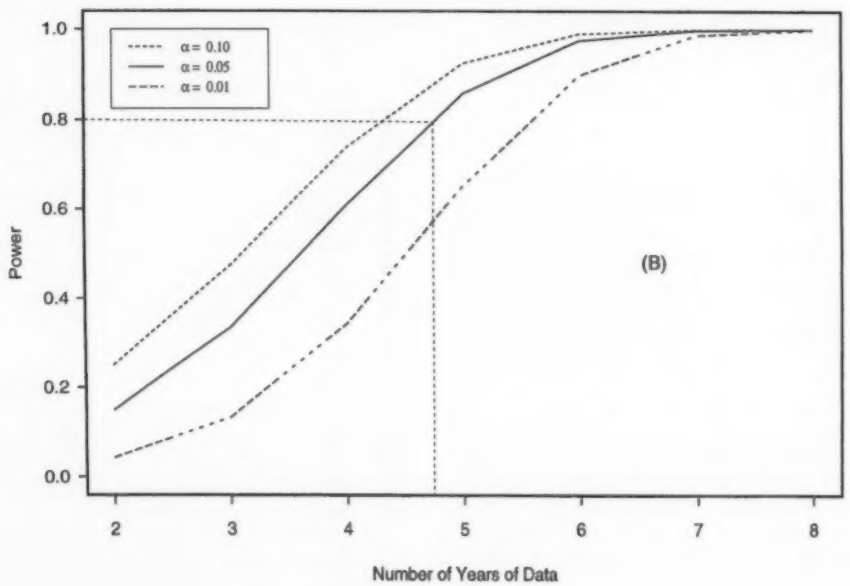
Fig. 6. Power curves for a 13-metric IBI with variances as given in Table 6 and a sample size of 18 streams. As indicated by the straight broken lines intersecting on the solid curve, for power = 0.8 and $\alpha = 0.05$, this IBI detects (A) a 7.7-unit (8%) year-to-year difference and (B) a 2-unit (2%) per year trend in mean IBI in 4–5 years. A higher power or lower α would require a greater IBI difference for detecting a year-to-year difference (Fig. 6A) or a greater number of years to detect a 2-unit per year trend (Fig. 6B).





Discussion

General IBI approach

Cairns et al. (1993) summarized several desirable indicator characteristics. An appropriate indicator is based on our ecological knowledge and conceptual models of ecosystems; it therefore incorporates elements of structure, composition, and function. It is also useful in waters other than those in which it was developed and is diagnostic, heuristic, or both. Finally, a good indicator has sufficiently small sampling and annual variability to be responsive to marked differences or changes in habitat quality and disturbance levels.

Our index of fish assemblage integrity has all the above characteristics. It was based on our ecological knowledge of the streams and their fishes, and the metrics included aspects of structure, composition, and function. It worked well on a different data set than that from which it was developed, and the individual metrics offered diagnostic and heuristic insights

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into the major stressors of concern and the probable consequences of continued landscape disturbances. The IBI's sampling and annual variabilities were low enough to allow detection of fairly subtle differences in physical and chemical habitat.

We successfully applied the general approach of Karr et al. (1986) for developing an IBI for streams of an Oregon agricultural region, despite its having no native fish species in common with streams from which they originally developed the IBI. Although we used only five of Karr's original 12 metrics, all but two others (hiders, filter feeders) were derived from the IBI literature. Six original metrics were replaced with more general metrics that can be used in waters where specific species or families are absent or rare. Such changes make IBIs more generally applicable in North America and the world (Hughes and Oberdorff 1998). Several original metrics were initially rejected as unsuitable from knowledge of the local fish fauna; all others were quantitatively evaluated. Candidate metrics that were tested and found highly redundant, unresponsive to physical and chemical disturbance gradients, or highly variable were not included in our final IBI.

Metric and IBI performance

Individual metric evaluations, if performed, are rarely reported. Although there are logical reasons to include each metric, those that are highly variable or unresponsive to physical and chemical habitat quality, like total number of individuals in this case, are best culled from the final index. Another option is to modify the sampling to reduce a source of error. For example, we found that percent filter feeders (lamprey) is particularly vulnerable to sampling error (Table 6), which can be reduced by carefully electrofishing their habitats. Despite selecting more general metrics for use in streams of the Willamette Valley ecoregion and elsewhere, we expect that some of our metrics may be problematic in other regions. We agree with J. R. Karr (University of Washington, Seattle, Wash., personal communication) that IBIs should be based on a set of nationally applicable metrics to the maximum degree possible. Scoring each metric from 0 to 10 and weighting metrics so that the IBI score ranges from 0 to 100 regardless of the number of metrics facilitates comparisons among different IBI scores across the continent or the globe. On the other hand, developing markedly different metrics and IBIs for each basin or ecoregion, instead of modifying scoring criteria or acceptable IBI scores, will likely complicate development and implementation of the IBI by management agencies.

The IBI was strongly correlated with the first principal component of a PCA of IBI metric scores. This suggests that a set of carefully selected metrics can be used in either an IBI or multivariate analysis approach to assess biological integrity, as long as those metrics are not redundant. Both approaches also require evaluation against independent measures of habitat quality or disturbance because IBI and PCA differences among sites may result from purely natural differences in fish species and their relative abundances. Also, both approaches require ecological knowledge; species relative abundances alone are insufficient. The question then is which approach most clearly and easily communicates relative biological integrity to a wide audience.

Our IBI effectively discriminated along disturbance gradients in an independent test using data of Friesen and Ward (1996). The IBI was also significantly correlated with a separate, integrated measure of chemical and physical habitat quality, indicating its sensitivity to a wide range of potential stressors. The relationship of the IBI to the minimum DO concentrations of sites sampled by Friesen and Ward (1996) is also encouraging. It indicates that our IBI was a useful indicator of biological integrity at sites other than those from which it was refined and was associated with DO, a common limiting variable that we did not measure when initially evaluating metric and index responsiveness.

In the development and evaluation of our IBI, we used quantitative biological, physical, and chemical data collected multiple times at multiple sites across an ecoregion. The need for such data may be obvious to many ecologists, but data sets similar to these are too infrequently collected by management agencies. Without multiple visits to a subset of sites, both among years and within the same season, we cannot evaluate critical components of variance and power. We need quantitative physical and chemical habitat data to estimate habitat quality in a meaningful manner and with sufficient precision (Ralph et al. 1994). Finally, we require quantitative, rigorously collected biological data if we intend to precisely assess the biological integrity of stream ecosystems, or to develop useful models of stream performance under varying levels of anthropogenic disturbance. For small Oregon streams, a sampling distance of 40–50 times the mean wetted width is needed to collect 90% of the species obtained by sampling a reach twice that length (L. Reynolds, Oregon State University, Corvallis, Oreg., unpublished data).

IBI statistical power

The variance among stream sites sampled accounted for the preponderance of variance in comparison with temporal and measurement variation (Table 6). This indicates that our IBI discriminated sites of varying biological integrity from the noise of measurement error and short-term temporal variation. Based on the relative sizes of the observed variance components, our IBI can detect an 8-unit (8%) year-to-year difference in mean IBI (for $\beta = 0.2$, $\alpha = 0.05$, $n = 18$) and a 2-unit (2%) per year trend in 5 years (Fig. 6). At the same α and β, Fore et al. (1994) reported a capacity to detect an 8.5-unit difference in mean scores, which is an 18% difference for an IBI with a range of 48 points. Such sensitivity differences between our IBI and that of Fore et al. (1994) may result partly from the larger and more variable database from which theirs was calculated. Our ability to detect such relatively small differences in mean scores reduces the probability of making type 2 errors with the IBI, i.e., failing to detect true differences.

Relationship between IBI variance and IBI scores

Previous researchers reported that IBI variance was greatest at low IBI scores (Karr et al. 1987; Fore et al. 1994; Yoder and Rankin 1995). Others have shown a positive correlation between IBI and its standard deviation, comparable change in IBI score at reference and disturbed sites, or no relationship between IBI score and IBI range or standard error. In our data, standard deviation was greatest at an intermediate IBI score and showed a weak increase with IBI score (Fig. 5). What might account for some of these differences? Karr et al. (1987) compared only two streams and based their covariance conclusion on sites within an individual stream; but they also

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reported that the stream with the higher quality habitat and IBI scores also had the greater standard deviation. Fore et al. (1994, fig. 6) demonstrated that the preponderance of the variability at one site with low IBI scores was associated with October sampling whereas the comparable summer samples were less variable than those associated with high IBI scores. Yoder and Rankin (1995) used coefficient of variation to portray variability; this statistic results from dividing the standard deviation by the mean. Although it clarifies relative standard deviation, dividing the same standard deviation by a low mean (e.g., IBI of 12) produces a fivefold higher coefficient of variation than dividing it by a high mean (e.g., IBI of 60). Therefore the apparent relationships between variance and low IBI scores previously reported in part result from a misleading statistic, sampling too few sites, or sampling when conditions are more variable. Wherever possible, these sources of variation should be eliminated so that they do not confound IBI variance linked with low or high IBI scores because the degree that a site varies is itself an important aspect of assessing its integrity.

Summary

In this paper, we demonstrated the use of an alternative to regional reference sites for estimating reference conditions and we modified Karr's IBI for use in Willamette Valley streams. Candidate metrics and the IBI were evaluated for responsiveness to varying degrees of chemical and physical disturbance; their variances were examined because studies were designated to estimate major variance components. We produced an IBI responsive to general types of disturbance and capable of detecting an 8% (8-unit) change in mean IBI scores in 1 year and a 2% (2-unit) per year trend in 5 years. This approach for developing and evaluating quantitative indicators to assess the biological integrity of aquatic ecosystems offers a model for indicator development in general.

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Discarding practices for commercial gadoids in the North Sea

Yorgos Stratoudakis, Robert J. Fryer, and Robin M. Cook

Abstract: Understanding fishers' discarding behaviour, and anticipating their reactions to changes in the biological or regulatory characteristics of a fishery, are important for dealing with the problem of discarding. In this paper, we investigate the discarding of haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), and Atlantic cod (*Gadus morhua*) in the North Sea, using data collected by scientific observers onboard Scottish demersal vessels. We describe discarding on each trip by species-specific discard curves and explore how these curves depend on biological and regulatory variables. There are large differences in the size of discarded fish between inshore and offshore areas, with offshore-operating vessels discarding larger fish (high-grading). Increases in legal landing size correspond to immediate increases in the size of discarded fish, particularly for haddock and cod in inshore areas. In general, discarding practices for haddock and cod are similar over time and consistent across gears, whereas decisions for the lesser valued whiting are more variable and can be affected by the catch composition.

Résumé : Si l'on veut régler le problème du rejet des poissons par les pêcheurs, il est important de comprendre le comportement des pêcheurs et de prévoir leur réaction face à la modification des caractéristiques biologiques ou réglementaires d'une pêche. Dans le présent article, nous examinons le rejet de l'aiglefin (*Melanogrammus aeglefinus*), du merlan (*Merlangius merlangus*) et de la morue en mer du Nord (*Gadus morhua*) à l'aide de données recueillies par des observateurs scientifiques à bord de bateaux écossais de pêche du poisson de fond. Nous décrivons les rejets effectués lors de chaque sortie à l'aide de courbes de rejets en fonction de l'espèce, et nous analysons dans quelle mesure ces courbes dépendent de variables biologiques et réglementaires. Nous observons des différences importantes entre la taille des poissons rejetés dans les zones côtières et ceux rejetés dans les zones du large : les bateaux travaillant au large rejettent des poissons plus gros (écrémage). L'accroissement de la taille légale des poissons débarqués correspond à des augmentations immédiates de la taille des poissons rejetés, en particulier de l'aiglefin et de la morue dans les zones côtières. En général, les rejets d'aiglefin et de morue sont similaires dans le temps et uniformes d'un engin à l'autre, tandis que les décisions relatives au merlan, espèce moins prisée, sont plus variables, et peuvent être influencées par la composition des captures.

[Traduit par la Rédaction]

Introduction

Discarding fish to the sea has for some time been accepted as an inevitable component of harvest fisheries. Recent estimates suggest that more than one quarter of all fish caught annually are wasted through discarding (Alverson et al. 1994). Such estimates help to appreciate the magnitude of the phenomenon and gradually lead to the situation where discarding is becoming a major management issue (Alverson and Hughes 1996). However, the decision to discard is taken on deck and is therefore largely a function of fishers' behaviour (Hilborn 1985). Understanding this behaviour and anticipating fishers' reactions to changes in the biological or regulatory characteristics of a fishery are important for dealing with the problem and reducing the waste.

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For unmarketable species the process is straightforward, because the entire catch is dumped overboard. For species with a commercial interest (target or bycatch), many factors can influence the final decision on whether to discard. These range from fisheries regulations and biological properties of the fished stocks to market forces and fishers' personal preferences (Crean and Symes 1994). The situation is particularly complicated in mixed-species demersal fisheries, where several considerations intermingle to create a complex and dynamic discarding behaviour (Pikitch 1991). Sampson (1994) and Gillis et al. (1995*a*, 1995*b*) applied ideas from behavioural ecology and microeconomics to elucidate aspects of the decision-making involved in discarding. However, these studies are based on analytical approaches that make minimal use of actual discards data.

In this paper, we investigate discarding practices using field data, rather than a theoretical approach. The data come from the Scottish discards sampling programme (Jermyn and Robb 1981), which sends scientific observers on commercial fishing trips. Whilst onboard, the observers monitor the fishing operation and obtain species-specific length frequencies of landings and discards. Here, we investigate how the proportions of each species discarded at length depend on various biological and regulatory variables. In particular, we examine the effect of fishing area and gear, quarter of the year, landing harbour, and the composition of the catch. Further, we examine temporal

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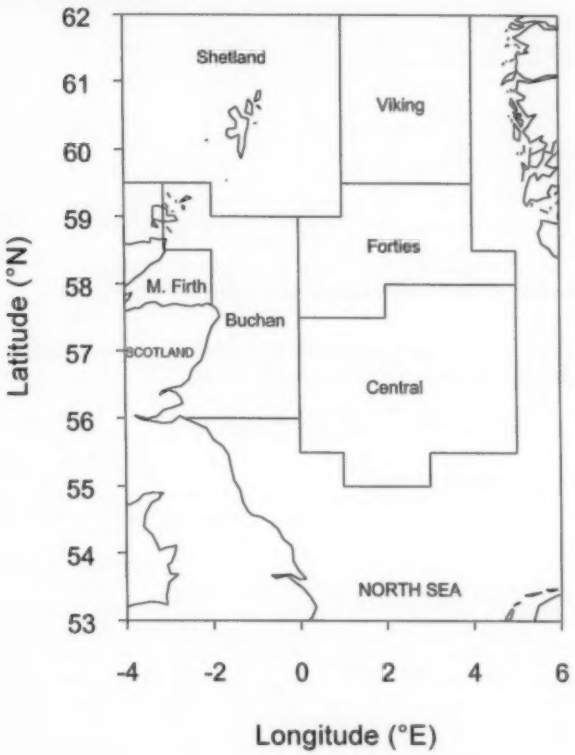
Fig. 1. Stratification of the North Sea for the Scottish discards sampling programme.



changes in the proportion discarded at length and associate them with changes in the management measures that prevail in the North Sea demersal fisheries.

We consider discarding by Scottish vessels using demersal mobile gears in the North Sea and focus on haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), and Atlantic cod (*Gadus morhua*), the three most important target species. The study covers the period 1975–1993, during which many aspects of the North Sea fisheries have changed (Symes 1992; Hislop 1996). These include biological changes, such as the reduction of some stocks to historically low levels, and regulatory changes, such as increases in nominal mesh size and minimum landing size.

Materials and methods

The Scottish discards sampling programme

Regular sampling of discards by scientific observers onboard U.K. vessels fishing in the North Sea and landing in Scotland began in 1975 and continues to the present day. The main objective of the programme is to provide annual estimates of the total quantities of the major commercial gadoids discarded in the North Sea (see Jermyn and Robb 1981). Cooperation in the programme by fishing vessels is on a voluntary basis.

The programme is based on stratified random sampling by area (Fig. 1), quarter of the year (January to March, etc.), and gear. Sampling concentrates on mobile demersal gears (trawlers and seiners) that are mainly used for catching gadoids and produce many discards in the process (Alverson et al. 1994). Resource limitations usually restrict sampling to one trip in each stratum where more than 10 000 h of fishing is expected (Jermyn and Robb 1981). As a result, the coverage is always small, ranging between 0.1 and 0.2% annually, both in terms of effort and landings. A sampling trip is defined to be the period spent away from the harbour until return for landing. This can vary from 1 day for small inshore vessels, to 2 weeks for larger vessels operating in the open sea. Sometimes, the fishing area or the operating gear change while the observer is onboard, and these are treated as separate trips.

Table 1 shows the number of trips sampled by area and gear. They are separated into the periods 1975–1984 and 1985–1993 to show how fishing methods, and hence sampling effort, have changed over the study period. Early sampling was concentrated on heavy trawlers (vessels larger than 27.5 m) and seiners. Light trawlers (vessels smaller than 27.5 m) became more important later, as fishing by heavy trawlers declined and many seiners converted to trawling. *Nephrops* trawlers (trawlers targeting the Norway lobster, *Nephrops norvegicus*) were first sampled in 1982, and pair trawlers (demersal trawlers operating in pairs) in 1986. In total, 982 trips on 242 vessels landing in 19 Scottish harbours were sampled during the study period, with an average of 23 h fishing observed per trip.

Discard summary statistics

While onboard, the scientific observers collect samples of the sorted catch to estimate discards and landings length frequencies of the three targeted gadoids (haddock, whiting, and cod). Numbers landed and discarded at length can be combined to calculate the proportion of the species catch discarded at length. Our aim is to explore, for each species in turn, how the proportion of fish discarded at length depends on biological and regulatory variables. To do so, we first describe the proportions discarded at length on each trip by two summary statistics. We then model the relationship between these summary statistics and the explanatory variables.

The proportions discarded at length can be thought of as coming from an underlying discard curve that relates the probability of discarding a fish to its length. The concept was introduced by Jean (1963) and is similar to that of gear selection curves (Wileman et al. 1996), only here the selection is imposed by the fishers, rather than the net. Intuitively, discard curves should be nonincreasing functions of length, and the data confirm this to be so; see Fig. 2, for example. Gear selection curves are usually summarized by two statistics that measure their location (length at which 50% of the fish are retained in the codend; L_{50}) and spread (the difference between the 75 and 25% retention lengths; interquartile range). In a similar way, we summarize the location of the discard curves by the species-specific discarding L_{50} (DL_{50}) and the spread by the discarding interquartile range. However, analysis of the discarding interquartile range did not reveal any systematic dependency on the explanatory variables (Stratoudakis 1997), and in the rest of this paper we concentrate on DL_{50}.

To estimate DL_{50}, we first have to fit a discard curve to the data for each trip. Techniques for estimating gear selection curves (Wileman et al. 1996) can easily be adapted for doing so. One option would be to fit a logistic curve (McCullagh and Nelder 1989). However, we fitted nonparametric isotonic curves that assume only that the curves are nonincreasing (Barlow et al. 1972; Millar 1993). The advantage of these curves is that they can describe both symmetric and asymmetric discarding patterns. A fitted isotonic discard curve is shown in Fig. 2. We then estimated DL_{50} by linearly interpolating between the fitted proportions of the isotonic discard curve. However, DL_{50} cannot be estimated when the species is not caught or when the entire species catch is discarded (particularly common in *Nephrops* trawlers where fish can legally be landed only in small amounts). Such trips were excluded from this analysis, resulting in slightly different data sets being used for modelling haddock, whiting, and cod DL_{50}s. Figure 3 shows summary box and whisker plots of the estimates of DL_{50} by species, excluding those from *Nephrops* trawlers. The range of

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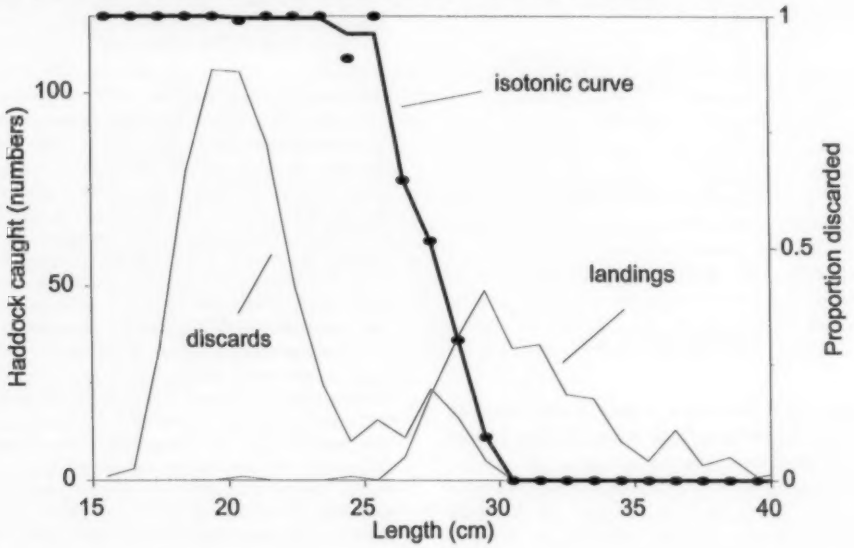
Table 1. Number of trips sampled by area and gear in the North Sea for 1975–1984 and 1985–1993.

	1975–1984					1985–1993				
Area	Heavy trawl	Seine	Light trawl	*Nephrops* trawl	Pair trawl	Heavy trawl	Seine	Light trawl	*Nephrops* trawl	Pair trawl
Shetland	38	61	8	—	—	4	37	19	—	21
Viking	—	28	—	—	—	1	25	2	—	2
Forties	—	24	—	—	—	—	24	6	2	5
Central	—	31	—	—	—	—	26	7	—	2
Moray Firth	—	41	11	18	—	—	36	22	52	2
Buchan	38	64	27	17	—	7	62	64	59	45
Other	8	1	2	—	—	2	17	8	3	1
Total	84	252	48	35	—	14	227	128	116	78

Fig. 2. An example of the methodology used to summarize the data from a sampled trip. Length frequencies for haddock discards and haddock landings from a seine trip in Buchan (June 1979), with the proportion of discards at length and the fitted isotonic curve.



estimates for cod is much wider than for haddock and whiting, possibly reflecting the scarcity of cod data.

We also estimated the standard error of each DL_{50} using bootstrap techniques (Efron and Tibshirani 1993). This can be thought of as measuring the "within-trip" variability associated with estimating DL_{50} and could be used later to correctly model the variance structure of the DL_{50}s in terms of within-trip and among-trip components of variation. However, exploratory analysis showed that, for haddock and whiting, the within-trip variability was much smaller than the among-trip variability and could effectively be ignored. The situation is less clear for cod (and for *Nephrops* trawlers), for which fewer fish are generally caught per trip, but for simplicity, we have ignored the within-trip component of variation throughout.

Explanatory variables

The explanatory variables available for this study can be conveniently grouped into three categories: catch variables measured during each trip, stratification variables associated with the selection of the vessel, and annual variables that change over time.

Catch variables

Detailed biological information is collected on each trip, so there are many ways of measuring the catch (i.e., discards plus landings) on that trip. We use the following, that seem to capture the broad features of the catch: (*i*) haddock catch per unit effort in kilograms per hour (HCPUE); (*ii*) whiting catch per unit effort in kilograms per hour (WCPUE); (*iii*) cod catch per unit effort in kilograms per hour (CCPUE); (*iv*) median length of species catch in centimetres (MED). Table 2 summarizes the distribution of these variables over the entire data set. The median length variable is species specific, in that the median length of the haddock catch is used to model haddock DL_{50} and so on. The CPUE and median length of the species catch are clearly measures of abundance and size of the species catch. The CPUEs of the other two gadoids are used to explore whether "species interactions" influence discarding. Biomass was estimated from the combined length distribution of discards and landings, using monthly length–weight relationships (Coull et al. 1989). Logarithms were taken to reduce the skewness of the CPUE distributions, with 0.5 added to each observation to allow for zero values (i.e., when the other two gadoids were not caught in the trip).

Stratification variables

These are the variables used to stratify sampling in the Scottish discards programme, with the addition of landing harbour: (*i*) gear

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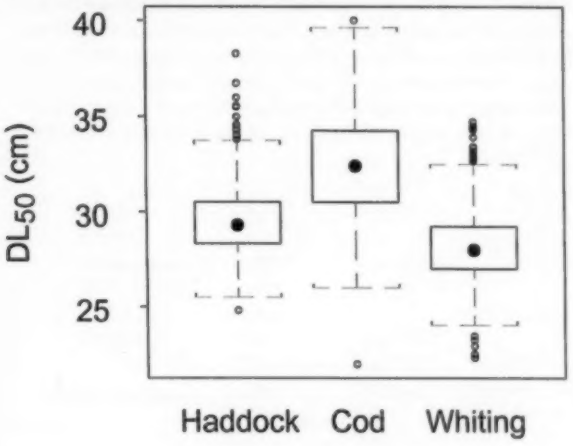
Fig. 3. Box and whisker plots of the estimated DL_{50}s, by species, excluding trips from *Nephrops* trawlers.



(see Table 1), (*ii*) area (see Fig. 1), (*iii*) quarter of year; and (*iv*) harbour. Note that all these variables are treated as categorical.

Annual variables

In addition to treating year as a variable in its own right, there are many variables that change only annually or every few years. These mainly relate to annually assessed biological parameters of the stocks (e.g., year-class strength, stock biomass, etc.) and to the prevailling management measures in the North Sea, i.e., minimum landing size (MLS), nominal mesh size (NMS), and quotas. MLS increased once within the study period, in 1979 for whiting (from 25 to 27 cm), and in 1989 for haddock and cod (from 27 to 30 cm and from 30 to 35 cm, respectively). NMS regulations were modified several times within the study period; the nominal size of meshes in the codend was standardized to 80 mm in 1980 and then increased to 85, 90, and 100 mm in 1987, 1989, and 1991 respectively.

It is obvious how changes in MLS may affect discarding decisions. Changes in NMS could affect discarding through changes in the size distribution of the catch; stock biomass and year-class strength through changes in the availability of resources and changing market conditions (Crean and Symes 1994); and quotas by limiting the biomass that can reach the market (Pikitch 1991). A summary of the biological variables related to the assessment of the North Sea gadoid stocks (e.g., spawning stock biomass and year-class strength) can be found in ICES (1996). Hislop (1996) discusses changes in the North Sea gadoid stocks within the study period, and Symes (1992) describes recent changes in the management regime for the North Sea demersal fisheries.

Unfortunately, having many annual variables presents modelling difficulties since, with 19 years' data, there are only 18 degrees of freedom available for investigating annual effects. To avoid problems of confounding and overfitting, we first use only year to explore temporal trends in discarding. We then relate significant year effects to other annual variables for a clearer biological interpretation.

Modelling DL_{50}

Our original intention was to use generalized additive models (GAMs: Hastie and Tibshirani 1990) to model DL_{50} for each species across the North Sea. GAMs generalize traditional regression techniques by relating the response variable to arbitrary, smooth functions of the explanatory variables. They are a particularly useful modelling tool, because they accommodate relationships of many different shapes, allowing the data to determine, to a large extent, the most suitable shape to be used. GAMs are being increasingly used in a fisheries context; see, for example, Swartzman et al. (1994) and Borchers et al. (1997). However, exploratory GAM fits revealed highly significant interactions between the explanatory variables, notably involving either area or gear. Since GAM software is not sufficiently developed to deal easily with such interactions, an alternative approach was required.

Table 2. Median and quartiles of each catch explanatory variable over the entire data set.

Variable	First quartile	Median	Third quartile
Haddock CPUE ($kg \cdot h^{-1}$)	6.6	10.8	16.3
Cod CPUE ($kg \cdot h^{-1}$)	3.0	5.6	9.0
Whiting CPUE ($kg \cdot h^{-1}$)	2.8	5.6	10.4
Haddock median length (cm)	26.2	29.1	31.3
Cod median length (cm)	32.2	36.2	42.3
Whiting median length (cm)	25.5	28.1	30.3

Our solution is to use two different regression techniques that examine the data from different directions. First, we use regression trees (Breiman et al. 1984; Clark and Pregibon 1993) as an exploratory tool to identify broad patterns in discarding across the North Sea. Regression trees are particularly suited to modelling interactions and dealing with a mixture of continuous and categorical explanatory variables (Venables and Ripley 1994). They operate by recursively splitting the data into homogeneous subsets (two at a time). Each split is determined by the value of an explanatory variable, with the data "below" the splitting value going into one group and the data "above" going into the other. The splitting point is chosen to minimize the within-group variance of the response variable in the two subsets.

We fitted a tree to the DL_{50} of each species using the explanatory variables described above:

$$DL_{50} \sim \text{Year} + \text{Area} + \text{Gear} + \text{Quarter} + \text{Harbour} + \text{HCPUE} + \text{CCPUE} + \text{WCPUE} + \text{Median}.$$

The trees were initially fitted with no constraints on the size of the fitted model, leading to some overfitting. The optimal tree size was then evaluated by cross-validation (Efron and Tibshirani 1993). The results of a regression tree must, in some sense, be regarded as exploratory, since there are no formal tests of significance involved in model selection. However, cross-validation gives the tree with the smallest estimated prediction error.

Second, we obtain more detailed insight into discarding patterns by fitting separate GAMs to subsets of the data, corresponding to each combination of area and gear. Using GAMs in this way avoids the problem of modelling the interactions involving area or gear. The drawback is that there is no formal way of assessing area and gear effects, other than by a qualitative inspection of the fitted models across areas and gears. However, we feel that combining the detailed GAM results with the "overview" provided by the regression trees allows us to built a cohesive picture of discarding patterns in the North Sea.

For each area and gear combination, we fitted a "full" model that included all explanatory variables (other than area and gear):

$$DL_{50} \sim \text{Year} + \text{Quarter} + \text{Harbour} + \text{HCPUE} + \text{CCPUE} + \text{WCPUE} + \text{Median}.$$

In this model, catch variables were fitted as smoothers with 4 degrees of freedom (df) and year was fitted as a categorical variable to allow DL_{50}s to vary freely between years. Normally distributed errors were assumed. The full model was then simplified using a stepwise selection procedure (Draper and Smith 1981), in which the catch variables were also considered as linear effects (1 df), and year as a smooth effect on 5, 3, and 1 df. Fewer degrees of freedom were allowed for year in area and gear combinations with less than 15 years of sampling. Model selection was based on Akaike's information criterion

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(AIC). However, in stepwise regression, terms nonsignificant at the 5% level may not be removed by AIC (e.g., Buckland et al. 1992). To overcome this, we did a backward search on the final model, removing all terms nonsignificant in an F test at the 5% level. The adequacy of the selected model was investigated by residual plots. Finally, we tried to replace any significant year effect by the other biological and regulatory annual variables.

Results

Figures 4*a*–4*c* shows the selected regression trees of haddock, cod, and whiting, respectively. The haddock and cod trees are very similar. Both show a strong spatial separation within the North Sea, with smaller DL_{50}s in Moray Firth and Buchan. This is equivalent to an inshore–offshore grouping, since Moray Firth and Buchan are coastal areas, easily approached from harbours in eastern and northern Scotland (Fig. 1). Both trees also show an increase in DL_{50} in inshore areas around 1989. This coincides with the only increase in the MLS of these species during the study period, although NMS also increased in the same year (from 85 to 90 mm). The haddock tree also shows that before 1989, vessels fishing inshore and landing in small harbours had smaller DL_{50}s than those landing in large harbours. Further, in offshore areas, haddock DL_{50} increases when larger haddock are caught. Possible explanations for these findings are discussed later.

The most important variable in the whiting tree is the median length of the whiting catch, with larger DL_{50}s when larger whiting are caught. There is also a temporal effect, with an increase in DL_{50} around 1979. Again, this coincides with the only increase in whiting MLS during the study period. After 1979, an area separation appears, with smaller DL_{50}s in Buchan and Shetland. Note that gear does not appear in any tree, which suggests that any gear effect can only be of secondary importance compared with those effects in the trees.

Tables 3–5 show the selected GAMs for each area and gear combination. Year is the most important explanatory variable, being highly significant in most data sets. The fitted temporal patterns are plotted in Figs. 5*a*–5*c* and show that DL_{50} generally changes in a smooth, linear, or stepwise manner over time. The temporal trends differ between areas and gears, demonstrating some of the interactions discussed earlier (i.e., between area, gear, and year). However, for haddock (Fig. 5*a*), common features can be observed in many data sets; DL_{50}s often show an increase from the late 1970s to the early 1980s, a peak around 1982–1983, a decrease during the rest of the 1980s, and another increase from 1989 onwards. The temporal patterns in cod (Fig. 5*b*) are simpler than in haddock and also show common features across data sets, with changes in DL_{50} often being adequately explained by MLS. For whiting (Fig. 5*c*), the temporal patterns are more ambiguous and suggest gear-dependent temporal trends. The most consistent is for seine (the gear most thoroughly sampled throughout the study), where the increase of MLS in 1979 generally coincides with an increase in DL_{50}.

For haddock and cod, the catch and stratification variables were sometimes significant (Tables 3 and 4), but these effects were not consistent either across areas (within gears), or across gears (within areas). Conversely, the whiting GAMs show an interesting relationship between DL_{50} and the catch variables. In offshore areas, there are strong relationships with the catch

Fig. 4. Regression trees for DL_{50}: (*a*) haddock; (*b*) cod; (*c*) whiting. Values in boxes are the fitted DL_{50}s at each level of the tree partition. Variables given in boldface are those significant for the ensuing binary partition. The levels of the significant variable that lead to different branches of the tree are indicated at each splitting point. For example, the first partition in the haddock tree is according to area. Trips in Buchan and Moray Firth have a fitted DL_{50} of 28.6 cm and trips in the remaining areas have a fitted DL_{50} of 30.4 cm.

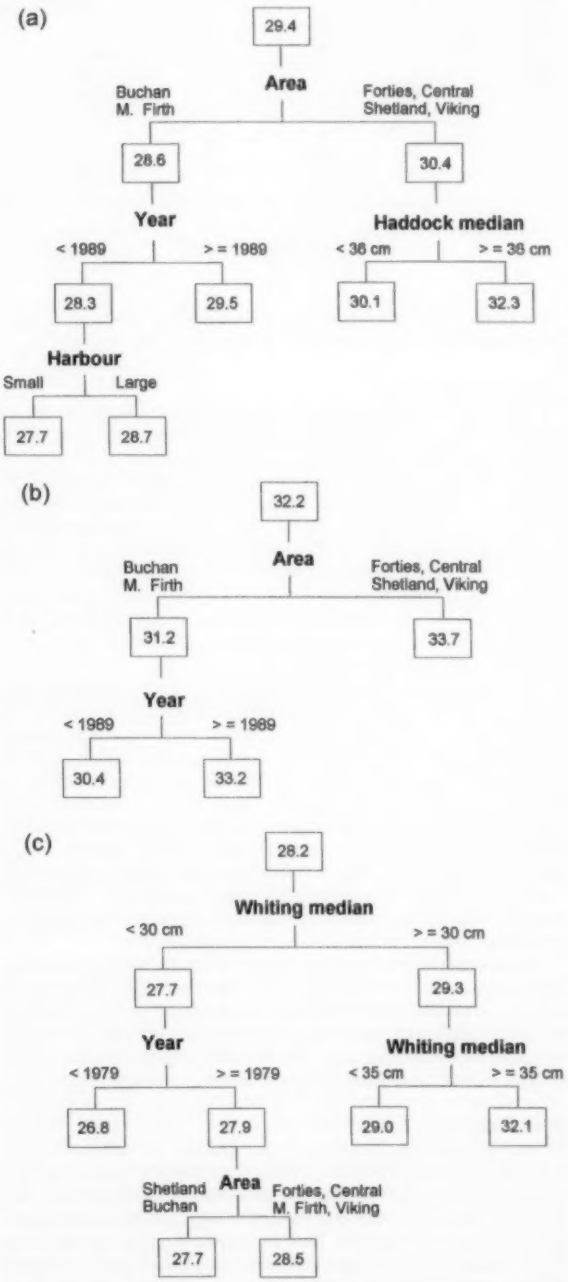

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Table 3. Selected model, residual variance (s^2), and proportion of explained variance (R^2) for GAMs fitted to haddock DL_{50} by area and gear.

		Selected model				
Area	Gear	Annual	Stratification	Catch	s^2	R^2
Shetland	Heavy	Year (3)	—	CCPUE +**HMED** (4)	1.1	0.74
	Seine	**Year** (5)	—	WCPUE (4)	2.1	0.43
	Light	—	—	CCPUE (4) + HCPUE + WCPUE	0.7	0.70
	Pair	Year	—	—	1.5	0.22
Viking	Seine	**Year** (5)	—	WCPUE + **HMED**	2.1	0.61
Forties	Seine	Year (5)	—	—	2.4	0.36
Central	Seine	Year	**Harbour**	CCPUE + **HCPUE** + HMED	1.1	0.61
Moray Firth	Seine	**Year** (5)	—	—	1.2	0.29
	Light	**Year***	—	—	0.4	0.81
	Nephrops	—	Harbour	CCPUE (4) + WCPUE (4)	1.3	0.44
Buchan	Heavy	Year*	—	HCPUE	1.3	0.59
	Seine	**Year** (5)	Harbour	HCPUE (4) + **HMED** (4)	0.6	0.71
	Light	**Year***	**Harbour** + quarter	**HCPUE** (4) + WCPUE (4)	0.4	0.78
	Nephrops	**MLS**	**Quarter**	—	1.2	0.69
	Pair	**Year**	—	CCPUE	0.9	0.56

Note: When an explanatory variable is fitted as a smooth term, it is followed by a value in parentheses that gives the degrees of freedom of the smooth. When year is fitted as a categorical variable, this is indicated by an asterisk. Only terms significant at the 5% level or greater are shown; those significant at the 1% level are underlined; and those significant at the 0.1% level are shown in bold. CCPUE, cod CPUE; HCPUE, haddock CPUE; HMED, haddock median length; WCPUE, whiting CPUE.

Table 4. Selected model, residual variance (s^2), and proportion of explained variance (R^2) for GAMs fitted to cod DL_{50} by area and gear.

		Selected model				
Area	Gear	Annual	Stratification	Catch	s^2	R^2
Shetland	Heavy	Year (2)	—	CMED	4.3	0.31
	Seine	Year (3)	—	WCPUE	5.2	0.25
	Light	—	—	—	5.2	—
	Pair	Year	—	—	5.0	0.29
Viking	Seine	Year (3)	Quarter	—	3.2	0.38
Forties	Seine	MLS	—	—	3.4	0.11
Central	Seine	MLS	Harbour	CCPUE (4) + HCPUE	1.8	0.53
Moray Firth	Seine	MLS	—	—	4.3	0.09
	Light	—	—	—	4.9	—
	Nephrops	—	Harbour	—	11.6	0.12
Buchan	Heavy	—	—	—	3.1	—
	Seine	**MLS**	—	—	3.0	0.49
	Light	MLS	—	—	2.8	0.31
	Nephrops	**Year** (3)	Harbour	**CCPUE** (4) + HCPUE	5.5	0.63
	Pair	**MLS**	Quarter	—	1.6	0.74

Note: When an explanatory variable is fitted as a smooth term, it is followed by a value in parentheses that gives the degrees of freedom of the smooth. When year is fitted as a categorical variable, this is indicated by an asterisk. Only terms significant at the 5% level or greater are shown; those significant at the 1% level are underlined; and those significant at the 0.1% level are shown in boldface. CMED, cod median length. Other variables are as in Table 3.

variables (Table 5). When significant, DL_{50} is always positively related to haddock CPUE, cod CPUE, and the median length of the whiting catch and negatively related to whiting CPUE. In inshore areas, there are few significant catch effects. These findings are interpreted below.

Discussion

Interpretation of patterns in DL_{50}

We now piece together the GAM and regression tree results to describe discarding patterns across the North Sea. The haddock and cod trees suggest an inshore–offshore effect on discarding (Figs. 4*a* and 4*b*). This is supported by the GAMs (Figs. 5*a* and 5*b*), where the predicted DL_{50}s are larger offshore. The GAMs show a similar effect for whiting (Fig. 5*c*). However, this is not supported by the whiting tree (Fig. 4*c*), where the median length of the (whiting) catch, rather than area, has the dominant effect on DL_{50}. This can be explained by considering how the median length of the whiting catch changes with area (Fig. 6*a*). Catches in Moray Firth and

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Table 5. Selected model, residual variance (s^2), and proportion of explained variance (R^2) for GAMs fitted to whiting DL_{50} by area and gear.

		Selected model				
Area	Gear	Annual	Stratification	Catch	s^2	R^2
Shetland	Heavy	—	—	**WMED**	2.3	0.34
	Seine	Year (5)	—	**HCPUE + WCPUE + WMED**	2.8	0.44
	Light	Year (3)	—	WCPUE + WMED	2.1	0.56
	Pair	Year	Quarter	CCPUE + HCPUE + WCPUE	1.4	0.79
Viking	Seine	**Year** (3)	—	CCPUE + WMED	2.4	0.62
Forties	Seine	Year (3)	—	—	3.3	0.23
Central	Seine	MLS	—	CCPUE + HCPUE + WMED	2.2	0.41
Moray Firth	Seine	**Year** (3)	—	HCPUE	1.4	0.58
	Light	Year	—	WCPUE	1.1	0.23
	Nephrops	Year (2)	—	WMED (4)	1.1	0.43
Buchan	Heavy	—	—	—	2.7	—
	Seine	**MLS**	—	WCPUE	1.4	0.13
	Light	—	—	CCPUE (4) + WMED	0.9	0.80
	Nephrops	**Year***	—	WCPUE	0.3	0.75
	Pair	Year	Quarter	—	0.7	0.47

Note: When an explanatory variable is fitted as a smooth term, it is followed by a value in parentheses that gives the degrees of freedom of the smooth. When year is fitted as a categorical variable, this is indicated by an asterisk. Only terms significant at the 5% level or greater are shown; those significant at the 1% level are underlined; and those significant at the 0.1% level are shown in bold. WMED, Whiting median length. Other variables are given in Table 3.

Buchan comprise smaller whiting than catches in offshore areas, so that area and median length of the catch are confounded. If we repeat the whiting regression tree excluding median length, then the inshore–offshore pattern emerges as the dominant effect. Thus, whiting DL_{50}s are greater offshore, where larger whiting tend to be caught. In fact, the median length of haddock is also related to area (Fig. 6*b*), suggesting that spatial patterns are confounded with haddock size. No such pattern is found for cod.

Table 5 suggests a further inshore–offshore difference in the discarding of whiting, since the catch variables have a large effect on whiting DL_{50} in offshore areas, but only a negligible effect inshore. In inshore areas, the median length of the whiting catch is distributed around the MLS (Fig. 6*a*), so discarding decisions are likely to be dominated by legal size considerations, rather than the composition of the catch. Offshore, whiting median length is distributed about sizes greater than the MLS, so the catch variables can become more important. In offshore areas, whiting DL_{50} tends to be positively related to the abundance of haddock and cod in the catch, and, unexpectedly, negatively related to the abundance of whiting in the catch. This can be interpreted as a shift in primary target species when whiting dominates the catch: a tight selection for the lesser valued whiting only occurs when whiting is abundant relative to the primary market targets, haddock and cod.

The GAMs show that temporal patterns in discarding can best be described by a smooth curve, a straight line, or a single step (Fig. 5). This confirms that DL_{50}s change systematically over time, rather than in response to noisy, short-term effects, such as recruitment variation. Only occasionally were the observed temporal patterns adequately explained by biological or regulatory annual variables, and this always involved MLS. Of course, this does not necessarily mean that biological and regulatory variables do not affect DL_{50}, merely that the changes in DL_{50} are more complicated that can be explained by these variables alone.

Changes in MLS offer a plausible explanation for many of the observed temporal patterns in DL_{50}. Even when MLS does not adequately explain all the temporal variation, increases in DL_{50} generally occurred around the time of MLS increases (Fig. 5). However, changes in NMS and MLS occurred at similar times. Although it is not possible to formally disentangle these two effects, there are several reasons why MLS is more likely to be the causative effect. First, NMS can only have an indirect effect on discarding decisions by altering the size distribution of the catch. Further, if NMS was the only factor to influence DL_{50}, it would have a simultaneous effect on all three species, which should lead to similar DL_{50}s across species. However, whiting DL_{50}s do not, in general, increase around 1989 (Fig. 5 c). Also, especially after 1989, the DL_{50}s of the three species are separated by several centimetres, lying close to their respective MLSs (Fig. 5).

On the other hand, for the MLS hypothesis to hold, MLS should have a similar impact across areas and gears. This is not evident in the regression trees, which suggest that the change in MLS only affected inshore areas. However, the GAM results suggest that haddock and cod DL_{50}s also increased in offshore areas around 1989 (Figs. 5*a* and 5*b*), and whiting DL_{50}s shifted in some offshore areas around 1979 (Fig. 5*c*). Before the changes in MLS, the DL_{50}s of all three species were consistently higher offshore. The shifts in MLS would therefore have been more easily accommodated offshore and, hence, might not have been identified by the regression trees.

The other temporal pattern to be explained is the increase in haddock DL_{50}s in the early 1980s and its subsequent decrease. Here, we can only speculate. The spawning stock biomass of haddock was comparatively high in the early 1980s but then reached historically low levels by the end of the decade (Hislop 1996), so DL_{50} could be related to species abundance. Also, the introduction of the Common Fisheries Policy in 1983 led to a tighter enforcement of quotas, so quota

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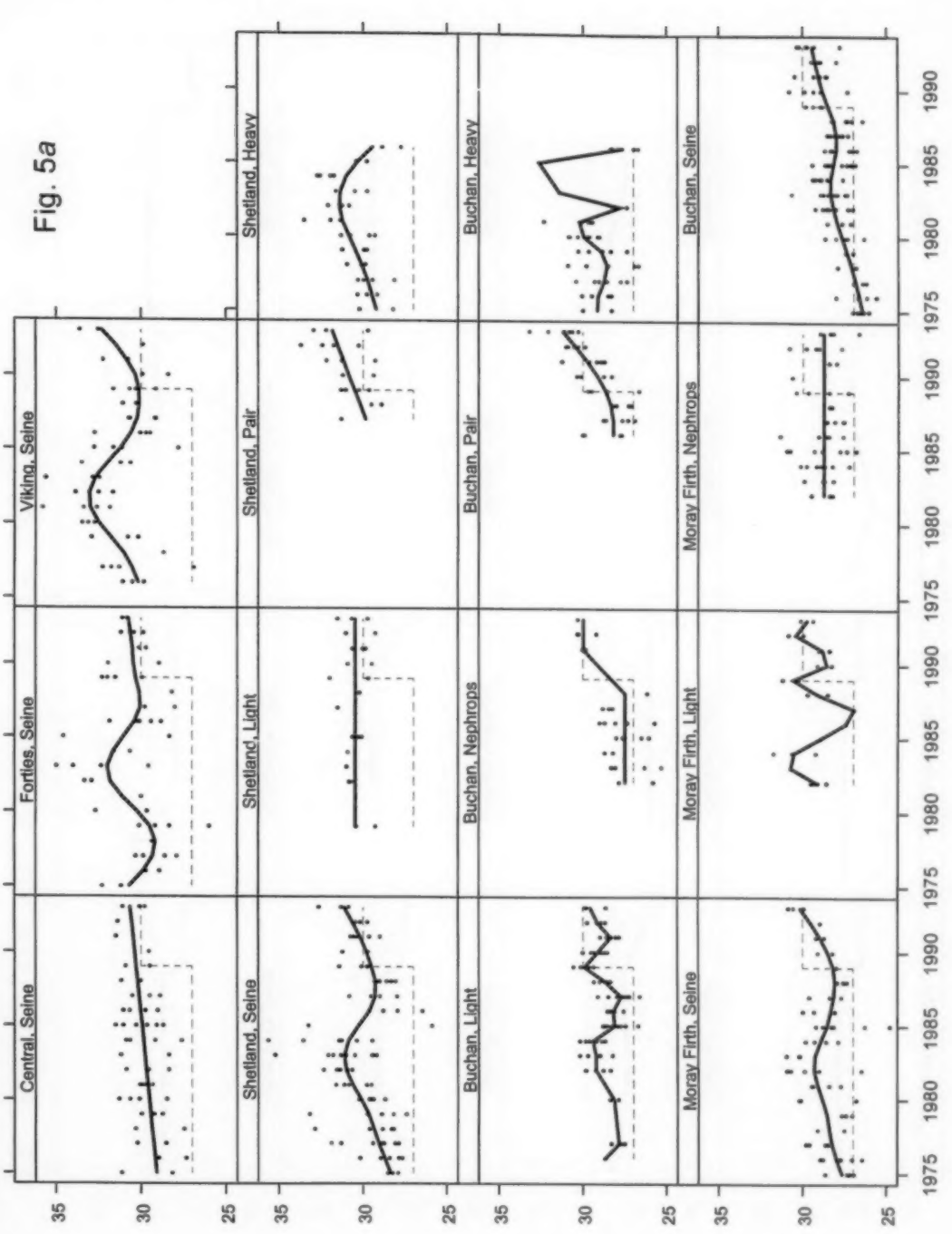

Fig. 5. Annual DL_{50} predictions and partial residuals by area and gear: (*a*) haddock; (*b*) cod; (*c*) whiting. The dotted line is the minimum landing size. The lower two rows correspond to inshore areas and the upper two rows to offshore areas.

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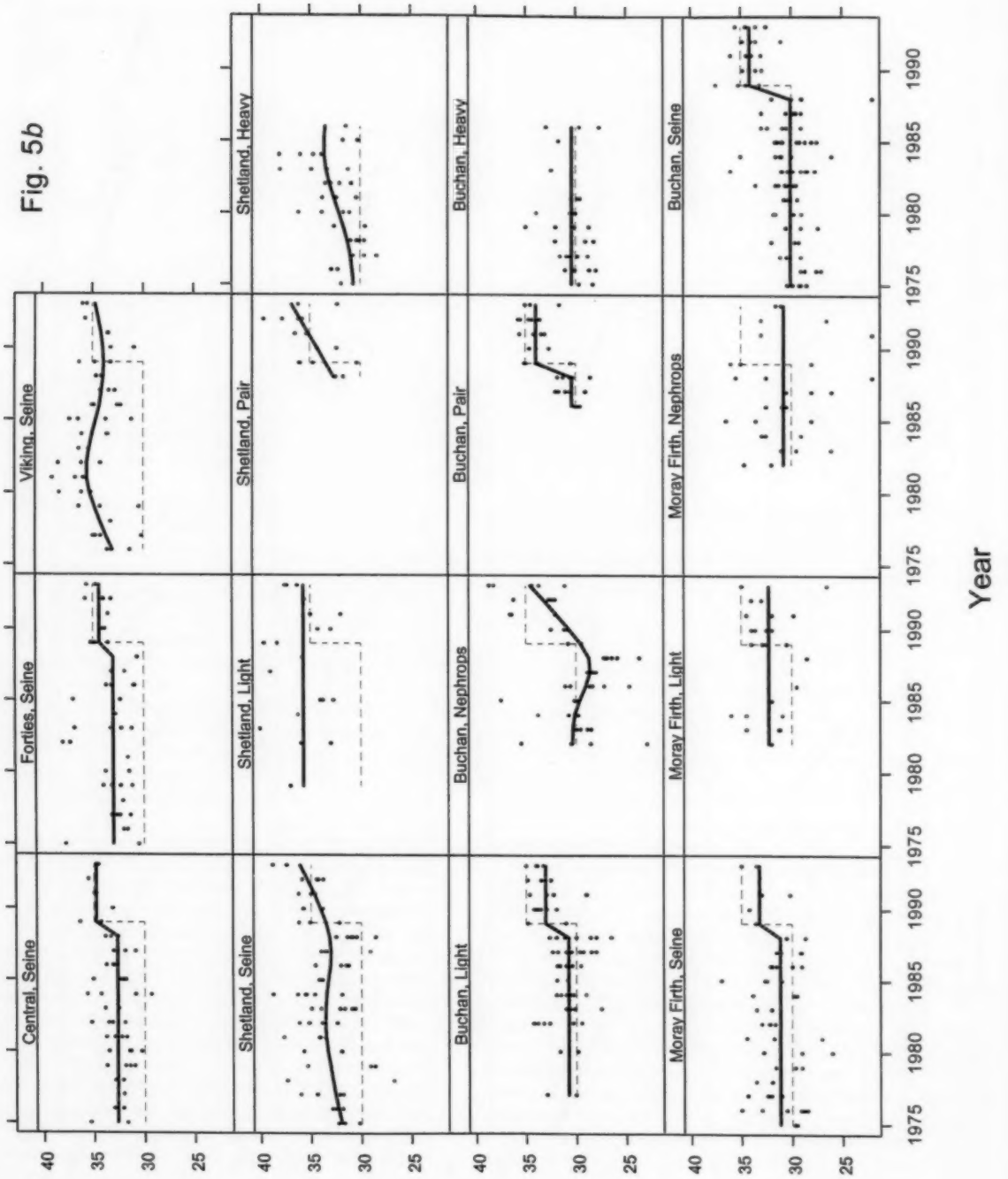

Fig. 5 (*continued*).

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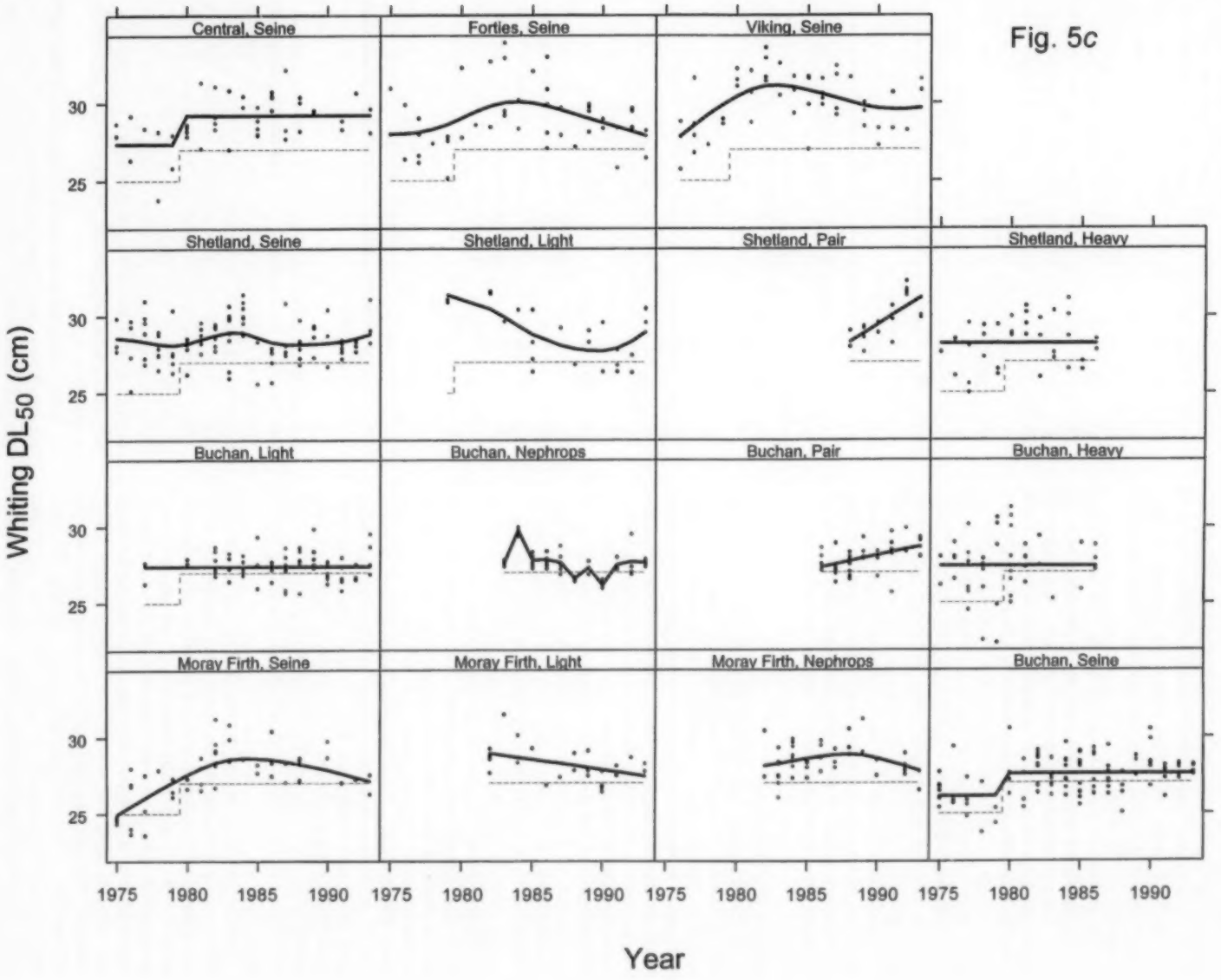
Fig. 5 (*concluded*).



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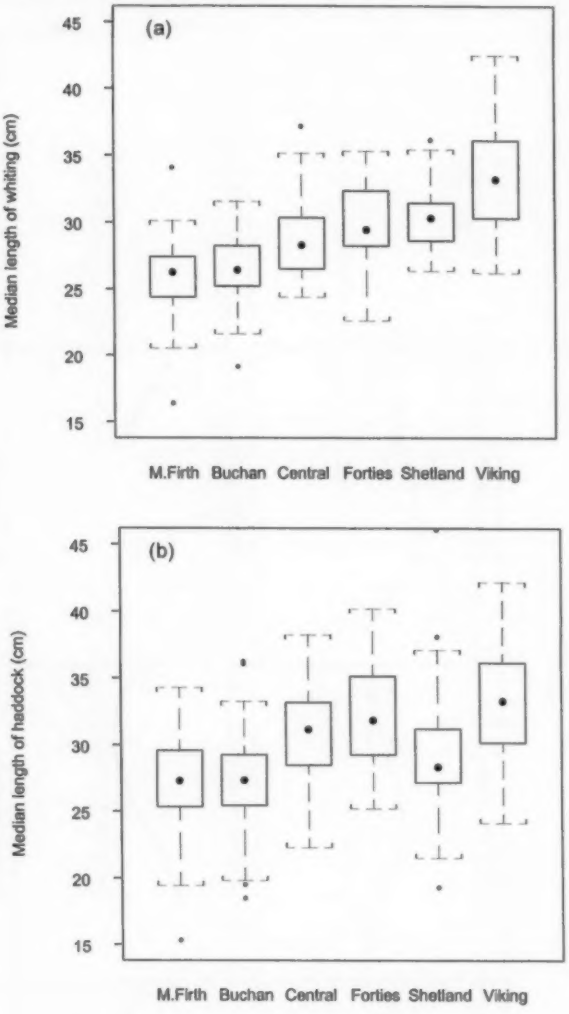
pressures could have started influencing discarding decisions around this time. When quotas can be easily reached, quota pressures might cause fishers to keep only the larger, more marketable, fish (Alverson et al. 1994). Haddock DL_{50}s were highest in a period (1982–1984) when landings were close to the allocated quotas, whilst the subsequent decrease in DL_{50} corresponds to a period when landings were much lower than the quotas (ICES 1996).

The preliminary GAM fits to the whole data set revealed highly significant interactions, particularly involving area and gear. We can interpret these interactions by comparing the fitted effects for each area and gear combination in Fig. 5 and Table 5. There are clearly important interactions between area and the catch variables for whiting, since there are strong relationships between DL_{50} and the catch variables offshore and very weak relationships (if at all) inshore. The temporal trends are also area and gear specific. Here, however, once we remove the main effect of larger DL_{50}s in offshore areas, the differences in temporal patterns between areas and gears are small and have no apparent structure. Thus, we conclude that, while these interactions are statistically significant, they do not represent important biological or technological effects.

Fig. 6. Box and whisker plots of the median length of whiting (*a*) and haddock (*b*) catches in seine trips by area.



General considerations

Despite the many vessels and trips sampled to provide the data for this study, generalizations should be considered with care. The data come from a voluntary scheme where sampling is restricted to vessels willing to cooperate and with working and living space available for the scientific observer. We therefore assume that discarding on these vessels is representative of Scottish demersal vessels as a whole. We also assume that fishing practices and discarding behaviour do not change in the presence of observers. Nevertheless, the Scottish data provide one of the best opportunities to investigate discarding on a relatively large spatial and temporal scale using real data.

The most striking result of this study is the difference in discarding practices between areas, conveniently summarized by an inshore–offshore separation. This separation is, of course, artificial, since there are no regulatory differences in the management of these areas. It is difficult to see why a vessel fishing "inshore" in eastern Buchan will adopt different discarding practices from one fishing "offshore," a few miles away in western Forties (see Fig. 1). However, this type of crude binary categorization is not uncommon when describing aspects of fishers' behaviour (Smith and McKelvey 1986) and is a useful tool for highlighting alternative discarding strategies.

If we divide the Scottish demersal fleet into an inshore and offshore component, larger vessels and more enterprising skippers ("stochasts" according to Allen and McGlade 1986) are likely to fish offshore, where there is a greater proportion of desirable fish sizes in the catch (P.J. Wright, Marine Laboratory, Aberdeen, U.K., personal communication). Such vessels tend to target larger fish, which have a higher market value. Anecdotal evidence suggests that, in the earlier years, vessels fishing offshore often used nets with mesh sizes larger than the NMS, to reduce the amount of "trash" fish in the catch and, hence, the time devoted to sorting. Before 1989, this practice led to the discarding of haddock and cod much larger than the MLS. This seems to indicate typical high-grading decisions. High-grading is the behaviour where potentially marketable fish are discarded to make space for more valuable fish when total landings are limited by trip quotas or hold capacity (Alverson et al. 1994; Gillis et al. 1995*a*, 1995*b*). In Scotland, hold capacity can hardly be considered restrictive, and quotas are mainly allocated on a vessel-specific monthly basis (Symes 1992). Nevertheless, it is plausible that a combination of quota pressures (albeit not imposed on a trip level) and financial considerations make fishers selective in what they keep for the market. After 1989, MLS considerations have become more important, with haddock and, especially cod, discarded close to the MLS.

Inshore areas are associated with smaller fish, but safer fishing conditions and reduced steaming time. Fishers less prepared to take risks ("cartesians" according to Allen and McGlade 1986) or with smaller vessels are therefore likely to fish closer to the home port. In these situations the scarcity of high-quality fish obliges fishers to land virtually everything

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above the MLS. Indeed, the selection is often so tight that measuring sticks are employed to compare fish with the imposed MLS. Thus, inshore fishers are mainly constrained by MLS, which has been shown to be a powerful enforcement tool generating an immediate reaction of the fishers. Note, however, that, despite the obvious effort to conform with the new regulation, even several years after the change in MLS for haddock and cod, many inshore gears still had average DL_{50}s below the legal size (Figs. 5*a* and 5*b*).

Whiting discarding strategies differ from those for haddock and cod. Again there is an inshore–offshore separation, with discarding inshore being mainly determined by the MLS. However, unlike haddock and cod, the increase in MLS was relatively easily accommodated by the fishers. Offshore, the composition of the catch has the greatest effect on discarding. A high-grading mechanism based on the threats of quota management is less likely for whiting, since landings are usually well below the allocated quotas (ICES 1996). A more plausible explanation is that whiting, having smaller commercial value than haddock and cod, is usually a secondary target when the other two species are abundant in the catch. In such cases, whiting are only kept at sizes comparable with those of haddock and cod, above the current whiting MLS. When whiting dominate the catch, fishers seem to switch target species and land most whiting of legal size. It might be possible to explore this idea further, by using optimal foraging theory models to investigate whether changes in the relative abundance of prey (catch of haddock, whiting, and cod) can adequately explain the observed selection strategy of the predator (fisher).

In conclusion, we have shown that data from routine discards sampling programmes can also address issues not directly related to the estimation of total discards. The identification of specific discarding practices (e.g., differences between the inshore and offshore sector, reactions to MLS changes), can help to evaluate the effectiveness and social implications of future management measures designed to curb discarding. Further, annual estimates of DL_{50} can be incorporated in assessment models for stocks where direct monitoring of discards is not available (e.g., Chen and Gordon 1997). Finally, our observation of illegal practices in sampled trips (landing below MLS) supports earlier views (Cotter 1995) that the reliability of discards sampling programmes depend on the development of a raport with the fishers, rather than on enforcement.

Acknowledgements

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The impact of global positioning systems and plotters on fishing power in the northern prawn fishery, Australia

Carolyn M. Robins, You-Gan Wang, and David Die

Abstract: The impact of global positioning systems (GPS) and plotter systems on the relative fishing power of the northern prawn fishery fleet on tiger prawns (*Penaeus esculentus* Haswell, 1879, and *P. semisulcatus* de Haan, 1850) was investigated from commercial catch data. A generalized linear model was used to account for differences in fishing power between boats and changes in prawn abundance. It was found that boats that used a GPS alone had 4% greater fishing power than boats without a GPS. The addition of a plotter raised the power by 7% over boats without the equipment. For each year between the first to third that a fisher has been working with plotters, there is an additional 2 or 3% increase. It appears that when all boats have a GPS and plotter for at least 3 years, the fishing power of the fleet will increase by 12%. Management controls have reduced the efficiency of each boat and lowered the number of days available to fish, but this may not have been sufficient to counteract the increases. Further limits will be needed to maintain the desired levels of mortality.

Résumé : À l'aide de données sur les captures commerciales, on a étudié les effets du système de positionnement global (GPS) et des systèmes de traceur sur la capacité de capture relative de la flottille crevettière du nord de l'Australie visant les crevettes tigrées (*Penaeus esculentus* Haswell, 1879, et *P. semisulcatus* de Haan, 1850). Un modèle linéaire généralisé a été utilisé pour prendre en compte les différences de capacité de pêche des bateaux et les variations d'abondance des crevettes. Les chercheurs ont constaté que, dans le cas des bateaux qui utilisent le système GPS seul, la capacité de pêche était supérieure de 4 % à celle des bateaux qui ne l'utilisaient pas. L'addition d'un traceur a augmenté la capacité de 7 % par rapport aux bateaux ne disposant pas de cet équipement. Pour chaque année entre la première et la troisième année pendant laquelle un pêcheur a travaillé avec des traceurs, on note une augmentation supplémentaire de 2 à 3 %. Il apparaît que, lorsque tous les bateaux seront munis d'un GPS et d'un traceur pendant au moins 3 ans, il y aura une augmentation de 12 % de la capacité de pêche de la flottille. Les mesures de gestion ont réduit l'efficacité de chaque bateau et le nombre de jours de pêche, mais il semble que cela n'ait pas été suffisant pour contrebalancer les augmentations. De nouvelles limites seront nécessaires si l'on veut maintenir le taux de mortalité visé.

[Traduit par la Rédaction]

Introduction

One of the main objectives in most fishery management plans around the world is to ensure the long-term viability of the fishery. To achieve this, the level of mortality or fishing effort that adversely affects fish resources must be known. There are two types of estimates of fishing effort: nominal fishing effort (the amount of resources devoted to fishing) and effective fishing effort (actual fishing mortality) (Cunningham and Whitmarsh 1980). Decisions based on nominal effort alone, or on inaccurate estimates of effective fishing effort, may result in management not meeting its biological objectives. Except where noted, we shall refer to "effective fishing effort" as "effort." In our example, nominal fishing effort is measured in fishing days. Nominal fishing effort may refer to any measure of resources devoted to fishing (e.g., fishing days, trawl hours, number of hooks, or number of trawl shots).

One of the causes of inaccurate estimates of effort is not taking into account increases in the effectiveness of each unit of nominal effort (Gulland 1956, 1969; Robson 1966; Taylor and Prochaska 1985). Such increases may occur quite rapidly when a new technological device or change in fishing method is found to help catch more fish or reduce the cost of fishing and is adopted by the whole fleet. Rothschild (1972), Griffin et al. (1977), Shepherd (1977), and Wang and Die (1996) discuss accurate estimates of fishing effort and why it is vital for a successful management plan.

The relative fishing power, a measure of a boats' effectiveness in catching fish compared with the standard boat in the fleet, can be analysed to determine what effect increases in effectiveness have had on nominal effort (Gulland 1956, 1969; Robson 1966; Beverton and Holt 1957; Sanders and Morgan 1976; Taylor and Prochaska 1985; Baelde 1991).

A collection of different fishing power models have been applied to many fisheries around the world. These techniques were used in the preliminary stock assessment of the New South Wales royal red prawn (*Haliporoides sibogae* de Man, 1907)(Baelde 1991). This study used several loglinear models to find the best combination of classification variables (effects of depth, latitude, and time period) and covariates (logarithms

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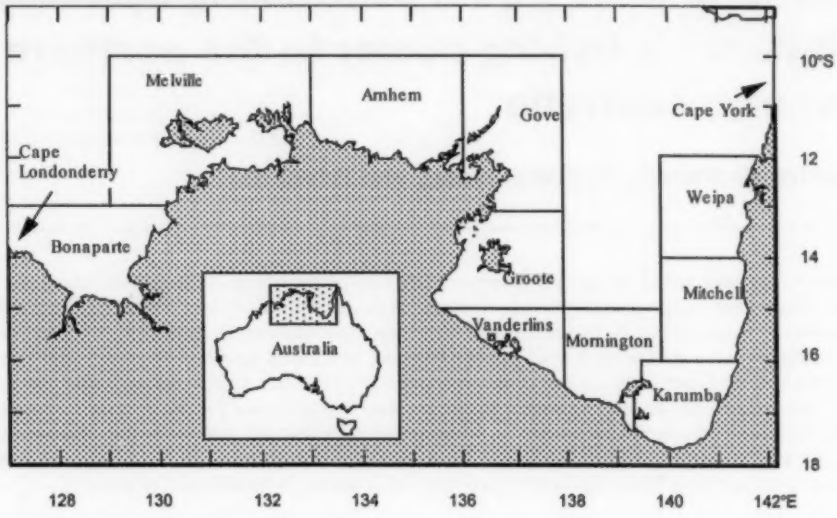
Fig. 1. Map of the northern prawn fishery, showing fishing regions used in our study.



of engine power, length, gross tonnage, headrope length, and codend mesh size of each vessel) that described the variability of catch per unit effort (CPUE). This information was used to estimate the CPUE of a standard vessel, which was then multiplied by observed effort to give a standardized effort and eventually a standardized CPUE. A fishing power study by Taylor and Prochaska (1985) examined the models behind planning an effective effort-limiting management strategy for the Gulf of Mexico reef fish fishery. This study used catch, vessel numbers, crew size, and vessel size in their loglinear model. The differences between the fishing power of adjoining states were determined, and the implications with respect to management were discussed. Brunenmeister (1984) also used a similar method in looking at the standardization of nominal effort for brown (*P. aztecus* Ives), white (*P. setiferus* (L.)), and pink (*P. duorarum* Burkenroad) shrimp stocks from the Gulf of Mexico. Similarly, changes in nominal effort was estimated using fishing power analysis in the western rock lobster (*Panulirus cygnus*) fishery, Australia (Brown et al. 1995). This study examined various factors influencing fishing power (radar, colour echo sounder, global positioning system, pot type) using a two-stage ANOVA regression technique. The nominal fishing effort was then standardized to determine changes in fishing power over time.

The northern prawn fishery (NPF), like all fisheries around the world, has experienced increases in effective effort. The NPF extends from Cape Londonderry, Western Australia, to Cape York, Queensland, along several thousand kilometres of coastline (Fig. 1). The average landing of export-quality prawns was around 8000 t/year from 1986 to 1994, which makes it one of Australia's most valuable fisheries (Dan et al. 1994). The total catch of 10 294 t landed by 125 trawlers in 1995 was made up of eight species of prawns. Three species constituted around 80% of this catch: the white banana prawn (*Penaeus merguiensis* de Man, 1888; 38%), brown tiger prawn (*P. esculentus* Haswell, 1879; 25%), and grooved tiger prawn (*P. semisulcatus* de Haan, 1850; 15%) (Robins and Somers 1994; Sachse and Robins 1995).

The NPF is divided into a daytime banana prawn fishery and a nighttime tiger prawn fishery, which have different fishing operations and different management strategies (Somers and Wang 1997). The highly variable annual catch of banana prawns appears to be closely associated with rainfall; the fishery does not appear to be overexploited (Somers 1994). In contrast, the catch in the tiger prawn fishery has declined since fishing effort increased in the early 1980s. This was not attributed to any one cause but nevertheless management acted quickly in an effort to reverse the downward trend. In 1986, management regulations were introduced to control fishing effort and thereby reduce overfishing of recruits and rebuild the stocks (Somers 1994). These regulations included a restructure, which reduced the fleet by around 50%; seasonal and area closures; daylight trawling bans; and net restrictions. However, there have been increases in the effectiveness of each fishing day since the establishment of this extensive management package, one of the most significant changes being the use of global positioning systems (GPS) and plotter systems.

The GPS and plotter units were introduced to the NPF fleet in 1988. This technology was quickly adopted across the whole fleet with most boats having a GPS and plotter by 1992. These devices changed how fishers catch prawns and opened new ground to trawling. As these navigational aids provided continuous positioning with more accuracy than previously achieved, fishing grounds could be trawled more effectively. The GPS uses satellites to establish the position of the boat, while the plotter enables the fisher to record information about the fishing operations on top of coastline charts. Prawn catches, untrawlable ground, "hot spots" (areas that have produced high catches), and any other navigational information are plotted on the chart. NPF skippers have expressed the opinion that GPS and plotter system have had a major impact on their fishing efficiency, particularly for the less experienced fishers.

We examined the effect that GPS and plotter systems have had on the relative fishing power of the boats in the tiger prawn

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fishery, which is the fishery most vulnerable to overfishing. Our approach is based on traditional fishing power models but allows for factors specific to this fishery: continuous recruitment and spatial and temporal differences in abundance due not only to different recruitment patterns but also to changes in fishing and natural mortality. This model permits catch to be a nonlinear function of effort and catchability to be a function of boat characteristics.

In the NPF, trawlers are not always skippered by the same person, so we have related fishing power to not only the boat but also the fisher. For this study we included the length of time a fisher has been using a plotter unit (referred to here as "fisher experience"). Fisher experience, gear size, and boat length have been included in this study as possible determinants of the fishing power.

The information obtained here will be used in all future stock assessments on the NPF. It will also be useful to help in reaching the objectives of the NPF management plan.

Materials and methods

Data sources

The analysis described in detail below is based primarily on commercial fishery data collected from trawler logbooks and landings returns. The daily logbooks include each trawler's catch in kilograms by species group (banana prawn (*P. merguinesis* de Man, 1888, and *P. indicus* Milne Edwards, 1837), tiger prawn, endeavour prawn (*Metapenaeus endeavouri* Schmitt, 1926, and *M. ensis* de Hann, 1850), and king prawn (*P. latisculcatus* Kishinouye, 1896, and *P. longistylus* Kubo, 1943) and the position in latitude and longitude where the greatest number of prawns were caught during that day. Processor returns collected from trawler owners and prawn processors were used to check and adjust if necessary the total logbook catch of each trawler. If the total logbook catch didn't equal the total processed catch then the correct landings for that boat was determined following consultation with the fisher and boat owner. An adjustment factor was used to scale up or down the total logbook catches to equal the total landings catch for the whole fleet.

Data from 1988 to 1992, were used, as these years span the introduction and general adaptation of the GPS and plotter system. The fishing seasons lasted from around 1 April to 30 November, but the main tiger prawn season was from 1 August to 30 November. Only the later period was used in this analysis, because most boats only target tiger prawns as these are the only abundant species during this period.

The NPF was divided into 10 regions: Weipa, Mitchell, Karumba, Mornington, Vanderlins, Groote, Gove, Arnhem, Melville, and Bonaparte (Fig. 1). As Mitchell and Bonaparte are predominantly banana prawn fishery grounds, they were omitted from the analysis.

All trawlers catch both banana and tiger prawns. The data set used, however, was the monthly catch (kilograms) and effort (days) per boat for each region when trawlers target tiger prawns. A boat was defined as targeting tiger prawns if over half the catch for that day was tiger, endeavour, or king prawns.

The analysis also includes vessel characteristics (boat length, headrope length of trawl gear, and the presence or absence of GPS and plotter units) as well as the number of years a fisher has worked with plotters. The vessel characteristics were recorded when the trawlers first entered the NPF and updated when necessary. Gear records are collected every year. The years that GPS and plotters were first installed in each trawler, and fisher lists for each year, were obtained from vessel inspection reports collected by Fisheries Patrol officers at the start of each season, a ships chandlery (Taylor Marine in Cairns), and during interviews with trawler owners and fishers.

The number of years the boat's fisher had worked with a plotter (fisher experience) was derived from fisher lists linked with GPS and plotter lists. From these data, each boat that fished each year was placed into one of five GPS categories:

(*i*) no GPS and no plotter and therefore no fisher experience,
(*ii*) GPS but no plotter and therefore no fisher experience,
(*iii*) GPS and plotter onboard and the first year the fisher has used a plotter,
(*iv*) GPS and plotter onboard and the second year the fisher has used a plotter, and
(*v*) GPS and plotter onboard and the fisher has three or more years experience with a plotter.

Statistical model

In general, using traditional methods as defined by Beverton and Holt (1957), in a fishery with a closed population (no emigration or immigration) the catch of each vessel is given by

$$(1)\quad C_{baym} = N_{aym}(1 - e^{-Z_{aym}})F_{baym}/Z_{aym} = F_{baym}\bar{N}_{aym}$$

where C_{baym} denotes the catch of the bth boat in area a during year y and month m; N_{aym} is the biomass at the start of month m; total mortality is denoted by Z_{aym}, and F_{baym} is fishing mortality. The average abundance during period m, $\bar{N}_{aym}$, replaces $N_{aym}(1 - e^{-Z_{aym}})/Z_{aym}$.

Fishing mortality is assumed to be

$$(2)\quad F_{baym} = q_{baym}E_{baym}$$

where q_{baym} is the catchability coefficient and E_{baym} is nominal fishing effort (in this case fishing days).

To account for generalizations that apply to a prawn fishery, including continuous recruitment, spatial heterogeneity, and temporal differences, we made the following assumptions in our model.

(A) The relationship between catch and abundance is

$$(3)\quad C_{baym} = F_{baym}h(N_{aym})$$

where $h(N_{aym})$ is an unknown abundance function. This is a generalization of eq. 1 to account for continuous recruitment, spatial differences, and temporal differences.

(B) Fishing mortality is

$$(4)\quad F_{baym} = q_{baym}(E_{baym})^{\delta}$$

where δ is an unknown parameter that generalizes eq. 2. This allows the possibility of CPUE dependence on nominal fishing effort (Taylor and Prochaska 1985; Richards and Schnute 1992). In general, the fishing mortality is assumed to be proportional to the nominal fishing effort. There are factors, however, that may violate this linear relationship: aggregation of stocks and targeting behavior of fishers. Our model allows a more general (nonlinear) relationship between catch and nominal effort.

(C) The catchability for each boat during year is assumed to be

$$(5)\quad \log(q_{b.y.}) = \alpha + \alpha_1\log(g_{b.y.}) + \alpha_2\log(l_b) + \beta_X$$

where α is the intercept, α_1 is the gear effect, $g_{b.y.}$ is the total headrope length of gear being used, α_2 is the length effect, l_b is the length of the boat, and β_X is the effect of the GPS category, where X is $X_{b.y.}$, the GPS category (000, 100, 111, 112, and 113) for that boat. Note that the GPS category 000 will be used as the baseline, and hence the parameter β_{000} is assumed to be 0 in the analysis. The other four GPS parameters reflecting the effects of GPS (relatively to category 000) will be estimated from the data.

The statistical model used for this analysis is obtained by combining eqs. 3, 4, and 5:

$$(6)\quad \log(C_{baym}) = \alpha + \alpha_1\log(g_{b.y.}) + \alpha_2\log(l_b) + \beta_X + \delta\log(E_{baym}) + \log(h(N_{aym})) + \varepsilon_{baym}$$

where ε_{baym} is the error term. The abundance term, $\log(h(N_{aym}))$, is expressed by area, year, and month, and all of their interactions to account for all possible changes in abundance.

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Table 1. Average headrope length, total headrope length, number of boats, and average boat length in the northern prawn fishery from 1988 to 1992.

Year	No. of boats	Headrope length (fathoms)		Boat length (m)	
		Mean	SD	Mean	SD
1988	222	23.24	4.58	21.80	3.12
1989	223	23.94	4.44	21.92	3.02
1990	200	23.66	4.52	21.60	3.09
1991	172	23.97	4.47	21.83	3.01
1992	170	23.43	4.55	21.48	3.05

While logarithmic transformation linearizes the model, it does not guarantee stabilization of the variance of the error components. To account for the heterogeneous variance components, a weighted regression was used. The weighting of nominal fishing effort was found to be appropriate in terms of stabilizing the residuals. The reasoning for this is that $\text{var}(\log(C)) \propto \text{var}(C)/C^2$, and if $\text{var}(C) \propto E$ and $C \propto E$, we would have $\text{var}(\log(C)) \propto 1/E$.

This model was fitted by PROC GLM in SAS, and the parameter estimates were used in the calculation of increase in fishing power due to the introduction of GPS and plotter units in the NPF for year:

$$(7) \qquad I_y = \beta_{100} d_{100,y} + \sum_{i=1}^{3} \beta_{11i} d_{11i,y}$$

where I_y is the average increase in fishing power resulting from the introduction of GPS and plotter units in the NPF, and $d_{x,y}$ are the corresponding proportions of boats in different GPS categories during year y.

The residual analysis confirms the theoretical result: the variance of $\log(C)$ is inversely proportional to the effort. We have also applied a Poisson loglinear model with overdispersion; the estimates are almost the same as the model used here. The overdispersion factor based on Pearson chi-square is 79, which is very high and indicates the importance of allowing overdispersion in the analysis. The corresponding confidence intervals taking account of overdispersion are also similar to those obtained here.

Results

Data summary

In 1988 and 1989 the GPS operated only sporadically, as few satellites were in orbit. It was not until 1990 that the system was useable most of the time and not until 1991 that the system was fully operational. During the first year (1988), 8% of boats had a GPS on board, and 7% had a GPS as well as a plotter; in 1989, these numbers rose to 31 and 20%, respectively; in 1990, they rose to 57 and 40%; in 1991, they rose to 97 and 87%; and in 1992, 99% of trawlers had a GPS and 98% had a GPS and plotter. This demonstrates how quickly technological innovations can spread in a fleet of vessels competing for a common resource.

The average headrope lengths of trawl gear used by NPF trawlers during the tiger prawn season did not change dramatically from 1988 to 1992. The average length of trawlers in the fleet also remained relatively constant from 1988 to 1992. The range of headrope length (12 to 28 fathoms; 1 fathom is 1.829 m) and boat lengths (12.8 to 30 m) did not change over the period of the study (Table 1).

Table 2. ANOVA results for the generalized linear model of relative fishing power in the northern prawn fishery.

Source	df	SS	MS	$P > F$
Year*	4	132	33	0.0001
Area*	7	84	12	0.0001
Year × area*	28	119	4	0.0001
Month*	3	194	64	0.0001
Year × month*	12	49	4	0.0001
Area × month*	21	109	5	0.0001
Year × area × month*	75	167	2	0.0001
log effort*	1	21 523	21 523	0.0001
log gear*	1	307	307	0.0001
log length*	1	81	81	0.0001
GPS*	4	67	16	0.0001
Model	157	32 055	204	0.0001
Error	5946	2 692	0.45	
Corrected total	6103	34 748		

*Type III sums of squares were calculated for these terms.

Table 3. Parameter estimates and their standard errors for the generalized linear model of relative fishing power in the northern prawn fishery.

Variable	Parameter	Estimate	SE
Effort (days)	δ	1.067	0.005
Average headrope length (fathoms)	α_1	0.566	0.022
Average boat length (m)	α_2	0.399	0.030
GPS but no plotter	β_{100}	0.037	0.010
GPS and plotter (first year of experience)	β_{111}	0.068	0.008
GPS and plottor (second year)	β_{112}	0.091	0.009
GPS and plotter (third or more year)	β_{113}	0.123	0.011

Statistical analysis

Estimates of model parameters, including the effect of the GPS and plotter systems on the power of boats, and the relative fishing power and fishing mortality estimates, were obtained using eq. 6 (Tables 2 and 3). The installation of a GPS without a plotter led to an increase of 4% in relative fishing power (over boats without a GPS). During the fisher's first year of using a plotter, fishing power increased by 7% over trawlers without a GPS or plotter; an additional year of experience with a plotter increased it to 9%; and a third year increased it to 12%. The coefficient for gear is 0.566, which means that, if the headrope length of the trawl gear increased from 16 to 25 m, the catch would increase by 22%. Similarly the coefficient for length of vessel was 0.4, or if the vessel length increased from 20 to 25 m, the catch would increase by 9%.

In our model, following Taylor and Prochaska (1985), catch is the observed variable and nominal effort one of the explanatory variables. In our case, nominal effort resulted in a good index of catch, with the estimate being close to 1 (Table 3). Therefore, it may be viable to use tiger prawn CPUE as an abundance estimate independent of effort. This may not be the case, however, for other fisheries. The generalized model that we used considers annual, monthly, and spatial differences in the interaction term. The differences in abundance between years, months, and regions (Fig. 2) are not considered here, as these are nuisance parameters for the purpose of our analyses.

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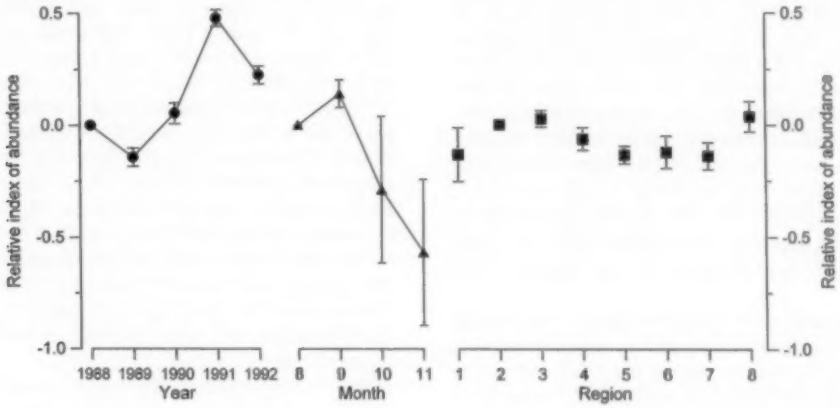
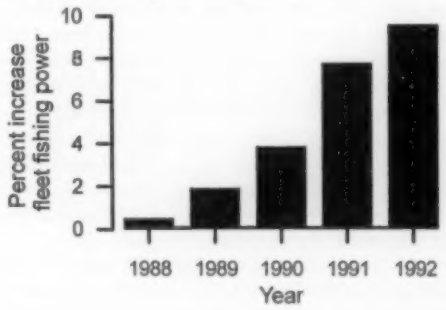
Fig. 2. Relative index of abundance for main effects, year (circles), month (triangles), and region (squares: 1, Weipa; 2, Karumba; 3, Mornington; 4, Vanderlins; 5, Groote; 6, Gove; 7, Arnhem; and 8, Melville). Note that all indices are relative to the standard year (1988), month (8), and region (2).



Baelde (1991), for example, includes interactions of the abundance term (depth, latitude, and time) in his model. Brunenmeister (1984) includes month and area terms but excludes all interactions, as he thought they would confound the estimates of area and month effects. Taylor and Prochaska (1985) omit the population variable and incorporate any changes in stock size in the error term. The model defined in Hilborn and Walters (1992, pp. 125 to 132) contains an abundance term that takes account of annual differences, and the authors note that the model can be changed to include interaction terms, such as vessel size and area interactions. Further work should investigate the usefulness of abundance estimates obtained from these types of analyses.

The annual increases in efficiency due to the use of GPS and plotters in the NPF as calculated using eq. 7 are indicated by a gradual climb from 0.5% in 1988 to 9.6% in 1992 (Fig. 3). By extension when all fishers have at least 3 years experience with a plotter, the increase is likely to be about 12%.

Fig. 3. Effect of the GPS and plotter system on the fishing power of the northern prawn fishery fleet from 1988 to 1992.



Discussion

The measurement of effort has always been a fundamental part of fisheries science, and it becomes even more important when fisheries are managed using effort limiting controls (input controls) (Rothschild 1972; Taylor and Prochaska 1985). The increase in efficiency of boats and fishers and, subsequently, fleets has occurred in fisheries around the world. This is due to better boat and gear design, improved fishing techniques, an increase in fisher experience, and an advancement in electronic technology. Every increase in efficiency results in nominal effort and effective effort diverging making the correct estimation of effective effort critical (Cunningham and Whitmarsh 1980).

The management objectives for the NPF are to ensure the long-term viability of the biological resource and the maximum economic efficiency of the fishery. The style of management is to regulate the level and pattern of fishing rather than the level or composition of landings (Taylor 1994). This fishery is no different from many others in that efficiency has increased dramatically since its discovery in the 1960s. In 1987, management aimed at lowering effort in the NPF by 40%. Has this been achieved? Nominal fishing effort has decreased considerably since the middle to late 1980s when more than 250 boats worked in the tiger prawn fishery. There are now only 124 boats fishing. We know that today's fleet is more effective than in the earlier days but not by how much.

Management strategies for the fishery cannot be evaluated unless the rate of increase in fishing power is known and the reasons for this increase understood. Tiger prawn predictions of equilibrium yield for the NPF are very sensitive to the rate of increase in fishing power that is assumed in the model. By varying the rate of increase in fishing power between 2 and 10% the model predicts that *P. esculentus* could be slightly underfished to severely overfished, while *P. semisulcatus* could be slightly underfished to fully fished (Wang and Die 1996).

The increase in fishing effort in the NPF from 1970 to 1986 was about 10-fold (Buckworth 1987). Over those years, nominal effort increased 430%; therefore, relative fishing power increased by 5% per year. However, Buckworth only took into account the rise in nominal days fished and increases in swept area resulting from increases in headrope length and engine power. He notes that, if other technological improvements had been included in the analysis, the increase would be even

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greater. The GPS and plotter system is one of these improvements. Not only has it revolutionized how the fishers catch prawns, but it has also opened new grounds to trawling.

The GPS and plotter units are not the only cause of increases in fishing power. Every change made to the trawlers increases fishing power, improves the lifestyle of the fishers, or increases the boat's profitability. In the last decade of fishing in the NPF, navigational and fish-finding aids have been introduced into the fishery, and gear and boats have been improved. Net makers are continually redesigning and modifying the gear, including the nets and the boards, chains, and setups. Boat builders and engineers are also making improvements. Fishers and crew are improving their fishing skills and techniques. It is impossible to calculate the increase resulting from these changes.

Interviews were held with 32 paid fishers, 6 fishers who own the boat, and 14 owners or company personnel to provide background information on the use of the GPS and plotter systems and describe how they used the system. Fishers were also asked to estimate how much its installation increased their efficiency of fishing. It was suggested that one of the main advantages of the GPS and plotter systems has been that the fisher can target more accurately. Once a productive area is detected, fishing is concentrated on that area until the catch drops significantly. The GPS and plotter system enable the fisher to keep the boat in the preferred area and even trawl over the same spot several times. Another advantage of the system is that information, such as untrawlable fishing grounds, can be recorded onto the charts. Areas that were previously ignored because of inaccuracies in the charted position of "foul ground" and of the boat can now be trawled to a greater extent. The fisher can trawl between and along reefs and around foul ground. Successful grounds from previous seasons are also recorded and fishers can find these grounds again. The trawlers and their gear sustain less damage as there are fewer "hook-ups," and less time is wasted searching for fishing grounds, so more time is spent trawling each night.

The fishing charts (plotter records) are continually improving. Many fishers believe that the GPS and plotter system has made fishing easier, and a fisher's experience is no longer such a large factor affecting boat profitability. Inexperienced fishers are now competing on a more equal footing with the more skilled. Similar behavior was suggested by Hilborn (1985), although he did not focus on the use of GPS and plotters, but states that, in general, it would be expected that many less skilled fishermen will imitate or follow the fishing pattern and techniques of the more skilled. This applies to a greater extent when they obtain copies of the plotter records of successful fishers. Many fishers freely share plotter records with friends, but most tend to work in loose groups often changing fishing partners. Eventually most plotter records are passed around the whole fleet. Consequently, there are no longer many "secret grounds," according to trawler fishers and owners.

Fishers from the previously mentioned interviews estimated that the GPS and plotter system had improved the fishing power of their trawlers by between 5 and 75%, with the most estimates falling between 10 and 40%. Our estimate is around 12% after 3 years. In the Western Australian rock lobster trap fishery, the use of GPS systems also increased fishing power by 12% (Brown et al. 1995). Our result is probably an underestimate, because boats without a GPS on board could follow boats with a GPS and plotter system.

Factors other than GPS and plotters also determine the fishing power of a boat, e.g., fisher skill (Gulland 1956; Cunningham and Whitmarsh 1980; Rothschild 1972; Brunenmeister 1984; Hilborn and Ledbetter 1985; Hilborn and Walters 1992). In fact, Gulland (1956) said that many trawler owners believe the fisher's efficiency is the most important factor in the fishing power of the boat, and the set of instruments used is the second most important factor. Such physical characteristics of a trawler as gear size, boat length, vessel tonnage, vessel age, and horsepower can also affect the fishing power of fleets (Beverton and Holt 1957; Rothschild 1972; Griffin et al. 1977; Hilborn and Walters 1992). There may have been technological changes other than GPS and plotters that occurred in the NPF fleet over the study period. There is no data available for these; therefore, their effect will be confounded with the abundance effects.

The "fisher effect" in the Icelandic cod fishery was found by Palsson and Durrenberger (1982) to be a myth. They concluded that the real reasons for a fisher's success or failure were the size of the boats and the frequency of trips.

In contrast, a fisher effect was apparent in the NPF. We have not estimated the variance accounted for by the fisher, but our analysis showed that fishing power increased by 2 or 3% each year from the first to the third year that a fisher has been working with a plotter unit. This can be attributed not only to fishers becoming more proficient with the system but also to an improvement in their fishing charts. The fisher builds up his own charts through experience and collects other fishers' charts to supplement his own. The rate at which the fisher's experience continues to increase beyond 3 years is not known but would be expected to plateau out after a length of time.

Although Buckworth (1987) only considered data from 1970 to 1986, his suggested 5% rate of increase in fishing power has been used in all recent NPF tiger prawn stock assessments (Somers 1994; Wang and Die 1996). As the GPS and plotter effect is only one component in the fishing power of a boat, the estimate obtained here will not change this accepted rate of increase but helps build a clearer picture of the sources for real effort increases in the NPF.

Nominal effort did drop 39% from 1988 to 1993 through a fleet size reduction as proposed by the management regime consisting of a voluntary and accelerated buy-back scheme and a compulsory surrender (Taylor 1994). However, the addition of GPS and plotter systems has increased the efficiency of trawlers by at least 12%, and other factors have increased it further. This increase equates to the addition of 15 boats into the current fleet of 126. If the estimate is conservative, it equates to even more additional boats. Management controls such as gear restrictions have reduced efficiency but probably not sufficiently to counteract the increase. Clearly a reduction in nominal effort does not necessarily mean a corresponding reduction in effective effort. It is obvious that, to successfully manage a fishery such as the NPF, all changes to the fleet that make the boats more efficient need to be documented and included in effort estimates.

This analysis has confirmed that technological change does impact fishing power. Technological improvements will continue to occur in most fisheries and consequently will lead to

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further fishing power increases. It is therefore essential that input-control fisheries, like the NPF, monitor technological change in their fleets.

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Nutrient–chlorophyll–Secchi relationships under contrasting grazer communities of temperate versus subtropical lakes

Asit Mazumder and Karl E. Havens

Abstract: Published data for total phosphorus (TP), total nitrogen (TN), chlorophyll (Chl), and water transparency (Secchi depth) for 420 lake-years in temperate North America and Europe and subtropical Florida were analyzed to test the hypothesis that subtropical and temperate lakes produce similar nutrient–Chl–Secchi relationships regardless of regional contrasts in seasonal, physical, chemical, and biological characteristics. We also tested the hypothesis that similarities in nutrient–Chl–Secchi relationships between temperate and subtropical lakes are expected only when the size or community structure of zooplankton remains constant. TP–Chl relationships were not significantly different for small zooplankton dominated temperate and subtropical lakes, but large herbivore (LH) dominated temperate lakes exhibited significantly lower Chl yield to TP than either subtropical small herbivore (SH) or temperate SH lakes. Although both nitrogen and phosphorus were important in determining the patterns and strengths of nutrient–Chl relationships in subtropical and temperate lakes, no particularly different role of TN or TP in determining Chl yield in the subtropical versus temperate lakes was observed. Unlike the nutrient–Chl relationships, Secchi depth seemed to decline as a function of both Chl and TP from subtropical SH to temperate SH to temperate LH lakes. Overall, the subtropical and temperate lakes seemed to produce similar general patterns of nutrient–Chl–transparency relationships, although mostly under the dominance of small zooplankton communities.

Résumé : Nous avons analysé des données tirées de publications sur la concentration de phosphore total (PT), d'azote total (NT) et de chlorophylle (Chl) et sur la transparence de l'eau (mesurée au disque de Secchi) de 420 années-lacs de la zone tempérée, en Amérique du Nord et en Europe, et de la zone subtropicale, en Floride, en vue de tester l'hypothèse selon laquelle les relations nutrients–Chl–transparence sont les mêmes dans les lacs des zones tempérée et subtropicale, malgré les différences que présentent les paramètres saisonniers, physiques, chimiques et biologiques d'une région à l'autre. Nous avons aussi testé l'hypothèse selon laquelle l'équivalence des relations nutrients–Chl–transparence entre les lacs tempérés et tropicaux ne peut être possible que si les effectifs ou la structure de la communauté zooplanctonique demeurent constants. Les relations PT–Chl ne différaient pas dans une mesure significative entre les lacs tempérés et subtropicaux où dominait une communauté de petits zooplanctontes; par contre, le rendement en Chl par rapport au PT dans les lacs tempérés où dominait une communauté d'herbivores de grande taille (GH) était de beaucoup inférieur à celui mesuré dans les lacs dominés par une communauté d'herbivores de petite taille (PH) tempérés ou subtropicaux. Les concentrations d'azote et de phosphore étaient des facteurs importants dans la configuration et les forces des relations nutrients–Chl des lacs subtropicaux et tempérés, mais rien n'indiquait que le NT et le PT remplissaient un rôle particulièrement différent dans le rendement en Chl dans les lacs subtropicaux par comparaison aux lacs tempérés. Contrairement aux relations nutrients–Chl, la transparence semblait diminuer en fonction de la concentration de Chl et du PT lorsqu'on passait des PH subtropicaux aux PH tempérés, puis aux GH tempérés. Dans l'ensemble, les relations nutrients–Chl–transparence semblaient avoir une configuration générale semblable pour les lacs subtropicaux et les lacs tempérés, mais il s'agissait surtout des lacs où dominait les communautés de petits zooplanctontes.

[Traduit par la Rédaction]

Introduction

Eutrophication of lakes, with excessive development of phytoplankton and associated deterioration of water quality, causes socioeconomic problems such as costly water purification for drinking, undesirable conditions for recreation, and massive fish kills due to lack of oxygen (Fulton and Paerl 1987). Consequently, extensive research has been conducted on the factors producing lake eutrophication and on the approaches for lake restoration (Jones 1987; review by Smith 1990). Factors regulating the biomass of phytoplankton as measured by chlorophyll *a* (Chl) concentrations and associated transparency of water have received considerable attention from aquatic ecologists (e.g., Schindler et al. 1971; Dillon and Rigler 1974; review by Smith 1990), and the prediction of Chl has been considered critical for successful lake management and restoration programs. In particular, knowledge about the form of nutrient–Chl relationships (e.g., Dillon and Rigler 1974; McCauley et al. 1989; Prairie et al. 1989) has allowed

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lake managers to establish nutrient (phosphorus and nitrogen) concentration and loading targets for temperate lakes. However, a large amount of variability in Chl is not accounted for by the concentration or loading of nutrients. To account for the high variance in average Chl yield at any given nutrient level (total phosphorus (TP) and total nitrogen (TN)), several physical, chemical, and biological characteristics of lakes have been considered (reviewed in Smith 1990), particularly fish predation and associated grazer communities (Pace 1984; Carpenter et al. 1985; Hansson 1992; Sarnelle 1992), grazer–nutrient interactions (Mazumder et al. 1988; Mazumder and Lean 1994), lake morphometry (Straskaba 1980), and thermal regimes (Riley and Prepas 1985; Levine and Schindler 1992).

Assuming that nutrients, grazers, grazer–nutrient interactions, lake morphometry, and thermal regimes all are important in determining phytoplankton biomass in lakes, Mazumder (1994*a*) developed models that accounted for these complex interactions in affecting TP–Chl relationships in temperate lakes. Because the presence of large species of *Daphnia* (a filter-feeding zooplankter) has been shown to be a key factor in regulating phytoplankton biomass in experimental studies, Mazumder (1994*a*) classified temperate lakes by the presence or absence of dense populations of large-bodied (>1 mm length) *Daphnia*. TP–Chl relationships developed for each group of lakes with contrasting grazer communities and thermal structure showed that robust predictive models can be developed if individual lake groups are analyzed separately.

These results have strong implications for lake management in temperate regions, but it is unclear to what extent they can be generalized to the subtropics, where lakes are experiencing excessive loads of anthropogenic nutrients (Ramberg et al. 1987; Havens and East 1997). It has been suggested that subtropical and tropical lakes differ in many ways from their temperate counterparts. They tend to be very shallow, with high densities of macrophytes (Canfield 1983), contain large inedible phytoplankton and small-bodied zooplankton (Crisman 1990; Crisman et al. 1995), support high densities of filter-feeding fish like gizzard shad (*Dorosoma cepedianum*) (Bachmann et al. 1996), which can graze both zooplankton and phytoplankton (Crisman and Beaver 1990), and are often suggested to be nitrogen limited. Perhaps for one or more of these reasons, certain researchers (e.g., Kratzer 1979; Canfield 1983) have reported TP–Chl relationships for subtropical lakes that differ from published temperate lake models (e.g., Dillon and Rigler 1974; Jones and Bachmann 1976). Nitrogen limitation of algal biomass seems to be more common in subtropical and tropical lakes (Henry et al. 1985; Dierberg et al. 1988; Davalos et al. 1989; Hecky et al. 1993), while phosphorus seems to be the primary limiting nutrient in temperate lakes (reviewed by Smith 1990). It is unclear whether differences in nutrient limitation or some other factors (zooplankton grazing) explain the different nutrient–Chl responses. Studies comparing temperate and subtropical lakes have not considered zooplankton community structure, although it is well established that subtropical lakes do not support large-bodied *Daphnia* (Crisman 1990). Thermal stress and high density of filter-feeding fish have been suggested as potential mechanisms preventing dominance of large grazers in subtropical lakes (Mallin and Partin 1989; Crisman 1990; Crisman and Beaver 1990; Crisman et al. 1995).

In addition to the nutrient–Chl relationships, it is also essential to evaluate the factors determining quality of lake water in terms of the clarity or transparency because several fundamental physical, chemical, and biological characteristics of lakes are dependent on water transparency. For example, transparency of water can influence the depth of algal photosynthesis (Carpenter et al. 1991), the concentration of hypolimnetic oxygen (Cornett and Rigler 1980; Mazumder et al. 1990*c*), and mixing and thermal structure (Yan 1983; Mazumder et al. 1990*b*; Mazumder and Taylor 1994). Both dissolved (dissolved organic carbon) and particulate materials can effect water transparency (Wetzel 1983). Among the particulate materials, although both inorganic clay particles and algae can reduce transparency, inorganic clay particles reduce transparency by absorbing a major fraction of incident light, while algae absorb only a fraction of light and backscatter a major fraction depending on the size distribution of algae (Kirk 1986; Mazumder et al. 1990*b*). As it has been suggested that suspended inorganic clay particles may be a dominant fraction of suspended particles in some of the subtropical lakes, it would be interesting to test if nutrient–transparency and Chl–transparency relationships for subtropical lakes differ from those for temperate lakes. We hypothesize that if the relative importance of clay particles to algae is greater in subtropical than in temperate lakes, the nutrient–transparency relationship should be steeper (greater slope) for subtropical lakes containing small herbivores (SH) than temperate SH lakes or temperate lakes containing large herbivores (LH). We also hypothesize that Chl–transparency relationships for subtropical and temperate lakes should be similar, but only among lakes with a similar community structure of grazers, however. Because the size of algae seems to be larger in lakes containing large grazers, especially under high concentrations of nutrients (Mazumder et al. 1988; McCauley et al. 1989; Mazumder 1994*b*), we expect to observe deeper Secchi depth per unit Chl and TP for temperate LH lakes than for either subtropical or temperate SH lakes.

The objectives of this paper are threefold: (*i*) to test that nutrient–Chl relationships for subtropical and temperate lakes are similar, although only under a similar structure of grazer communities; we expect that TP–Chl relationships for subtropical lakes should produce intercepts and slopes similar to small zooplankton dominated temperate lakes, but not to large grazer dominated temperate lakes; (*ii*) to test that if subtropical lakes are more predominantly nitrogen limited, while temperate lakes are more predominantly phosphorous limited; we expect stronger TN–Chl relationships for subtropical lakes, while we expect to observe stronger TP–Chl relationships for temperate lakes; and (*iii*) to test that subtropical and temperate lakes produce similar TP–Secchi and Chl–Secchi relationships, again, only for small zooplankton dominated lakes; our expectation is based on the hypothesis that processes or factors determining phytoplankton biomass and water transparency do not differ in subtropical versus temperate lakes. In this paper, we demonstrate that large grazer dominated temperate lakes are distinct outliers in comparative models for subtropical and temperate lakes in terms of nutrient–Chl or nutrient–Chl–Secchi relationships.

Materials and methods

Chl–nutrient–transparency relationships were obtained from two

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multilake data sets: (*i*) 361 lake-years ranging from ultraoligotrophic to hypereutrophic temperate lakes from a variety of regions in North America and Europe (Mazumder 1994*a*) and (*ii*) 59 subtropical lakes in subtropical Florida (Bachmann et al. 1996). The original data set for subtropical lakes had 60 lakes. Data for one of the lakes was not used because it had >1000 μg TP·L^{-1}, three times more than the maximum concentration among the remaining 59 lakes. Secchi depth data were available for a subset of the original data for temperate lake ecosystems, but were not presented in Mazumder (1994*a*). Details regarding the data and their methods of collection and analyses may be found in these two papers (Table 1). Note in Table 1 that for temperate lakes the data for the four variables TP, TN, Chl, and Secchi were not available for all the lakes. A subset was used for TN–Chl, TP–TN–Chl relationships ($n = 93$ for temperate SH lakes, $n = 50$ for temperate LH lakes). Similarly, for TP–Chl–Secchi relationships, 85 and 40 data points were available for temperate SH and LH lakes, respectively. In brief, data on TP, TN, Chl, and Secchi for the assembled set of lakes are based on epilimnetic samples collected during the summer season, at biweekly to monthly intervals (temperate lakes) or on three occasions per lake during summer (Florida lakes). In the analyses presented here, the data have been averaged to provide summer seasonal means.

Temperate lakes were classified as being dominated by SH or LH to indicate the absence or presence of substantial populations of the large-bodied *Daphnia* taxa described above (for criteria, see Mazumder 1994*a*). Although zooplankton were quantified for the subtropical lakes, only coarse-scale information (numerical densities of total copepods, total cladocerans, and total rotifers) was obtained (Canfield and Hoyer 1992). Nevertheless, it has been well established in a large number of studies of lakes in Florida and subtropical lakes in general (Fernando 1980) that none of the large-bodied *Daphnia* taxa occur (review by Crisman 1990). The only native daphnid, *Daphnia ambigua*, attains a size considerably below 1 mm and typically is absent from the plankton during the summer season, perhaps as a result of stress induced by high water temperatures (Mallin and Partin 1989; Crisman et al. 1995; Beaver and Havens 1996). Therefore, all of the subtropical lakes considered here are effectively classified as SH systems. Mazumder (1994*a*) also classified the temperate lakes on the basis of thermal criteria, differentiating between mixed and stratified systems. However, because detailed thermal profiles were not reported for the Florida lakes, we did not consider this potentially important variable in our analyses and concentrated on grazer contrasts for both temperate and subtropical lakes.

Statistical analyses

Data were analyzed using Systat version 5.1 (Wilkinson 1991). Linear least square regression models were developed first to produce results comparable with those published previously. Errors for slopes and intercepts in all the least square regression equations were expressed as standard errors of means. Statistical differences among linear models for LH temperate, SH temperate, and SH subtropical lakes were tested by comparing slopes and intercepts. Multivariate regression analyses also were performed to evaluate the importance of additional independent variables, such as TN, in explaining the variance remaining unexplained by the TP–Chl relationship. The same statistical analyses were done to test the TP–Secchi and Chl–Secchi relationships for temperate and subtropical lakes. Because the Chl–nutrient relationships displayed pronounced nonlinearity (e.g., sigmoidal patterns), we also developed locally weighted sequential smoothing (LOWESS) algorithms. Similar methods have been used by others (McCauley et al. 1989; Prairie et al. 1989; Mazumder 1994*a*). The purpose of developing LOWESS patterns was only to demonstrate that sigmoidal patterns were common for both subtropical and temperate lake ecosystems. Third-order polynomial equations were developed to test if the sigmoidal patterns were the best fits for TP–Chl relationships.

To test the significant differences in TP–Chl, TN–Chl, TP–Secchi, and Chl–Secchi relationships for temperate SH versus subtropical SH lakes and for temperate LH versus subtropical SH lakes, we conducted ANCOVA with the two regions as two categories and independent variables as covariates. A significant probability for the effect of category suggests significantly different *y*-intercepts, and a significant probability for covariate × category interaction suggests significantly different slopes.

Table 1. General characteristics of temperate and subtropical lakes.

	Temperate SH	Subtropical SH	Temperate LH
TP			
Mean	13 (235)	17 (59[a])	24 (126)
Maximum	991	384	995
Minimum	1	1	3
TN			
Mean	300 (93)	641 (59)	393 (40)
Maximum	2302 (3)	793	2147
Minimum	69	82	66
TN:TP			
Mean	48.8 (93)	47.1 (59)	16.1 (40)
Maximum	180.8	205.8	70.4
Minimum	6.4	2.3	2.2
Chl			
Mean	6 (235)	9 (59)	4 (126)
Maximum	511	241	219
Minimum	0.6	1	0.20
Chl:TP			
Mean	0.72 (235)	0.65 (59)	0.11 (126)
Maximum	3.75	1.88	0.41
Minimum	0.11	0.06	0.01
Secchi			
Mean	1.40 (85)	1.52 (59)	2.99 (40)
Maximum	9.70	5.79	11.99
Minimum	0.30	0.30	0.60

Note: Numbers in parentheses indicate the number of lake-years of data analyzed for each variable. All data are seasonal means. TP, μg·L^{-1}; TN, μg·L^{-1}; Chl, μg·L^{-1}; water transparency indicated by Secchi depth (m); SH, small herbivore dominance; LH, large herbivore dominance. Data sources: temperate lakes, references cited in Mazumder (1994*a*); subtropical lakes, Bachmann et al. (1996).

[a]The original data set for subtropical lakes had 60 lakes. We excluded one lake because TP was 1000 μg·L^{-1}.

Results and discussion

TP–Chl relationships

The least square regression equations for TP–Chl relationships (Fig. 1) were as follows.

Subtropical SH lakes:

(1) $\log \text{Chl} = -0.36 \pm 0.09 + (1.06 \pm 0.07)\log \text{TP}$; $n = 59$, $r^2 = 0.79$, $P < 0.001$.

Temperate SH and LH lakes combined:

(2) $\log \text{Chl} = -0.35 \pm 0.05 + (0.94 \pm 0.03)\log \text{TP}$; $n = 361$, $r^2 = 0.71$, $P < 0.001$.

Temperate SH lakes:

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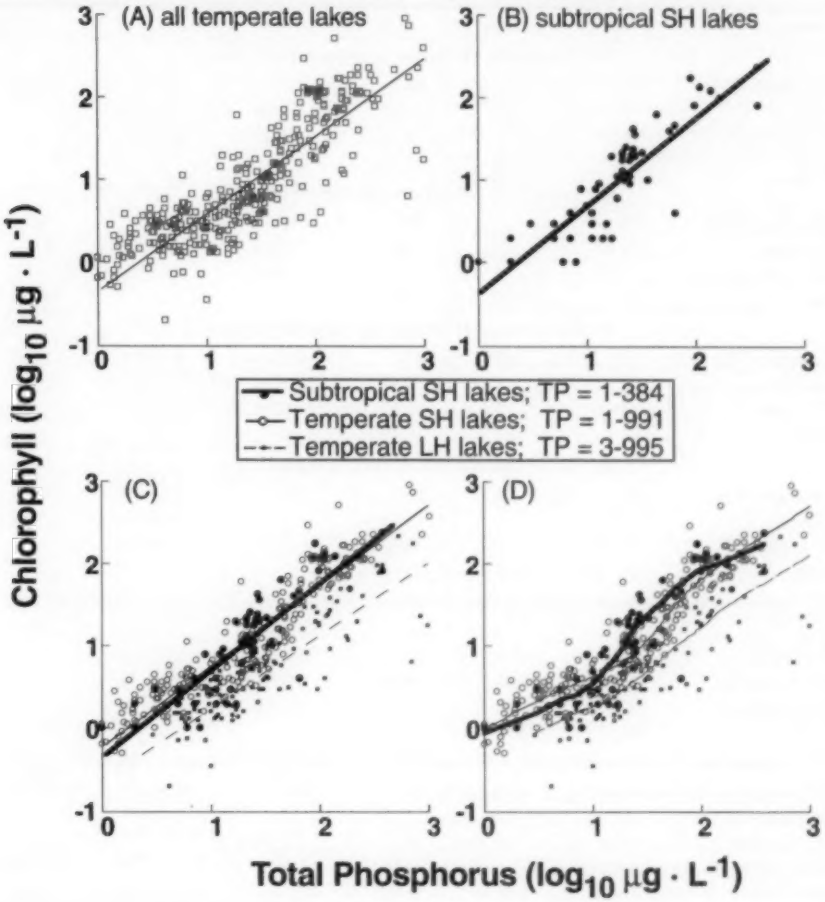
Fig. 1. Relationship between TP and Chl in (A) temperate SH and LH lakes combined and (B) subtropical SH lakes and (C) linear relationships and (D) LOWESS smoothing curves for subtropical SH (solid circles, thick solid line), temperate SH (open circles, thin solid line), and temperate LH (solid squares, broken line) lakes. The appropriate equations are presented in the text.


(A) all temperate lakes
(B) subtropical SH lakes
Subtropical SH lakes; TP = 1-384
Temperate SH lakes; TP = 1-991
Temperate LH lakes; TP = 3-995
(C)
(D)
Chlorophyll (log10 µg · L-1)
Total Phosphorus (log10 µg · L-1)

(3) $\log Chl = -0.21 \pm 0.03 + (0.97 \pm 0.02)\log TP$;
$n = 235$, $r^2 = 0.87$, $P < 0.0001$.

Temperate LH lakes:

(4) $\log Chl = -0.60 \pm 0.07 + (0.87 \pm 0.05)\log TP$;
$n = 126$, $r^2 = 0.73$, $P < 0.001$.

Subtropical SH lakes (eq. 1), temperate SH and LH lakes combined (eq. 2), and temperate SH lakes (eq. 3) produced relationships with *y*-intercepts and slopes that were not significantly different from each other (ANCOVA, Table 1). However, TP explained greater variance for subtropical SH lakes ($r^2 = 0.79$) than that for temperate SH and LH lakes combined ($r^2 = 0.71$). To demonstrate that TP–Chl relationships for subtropical and temperate lakes are similar only under the dominance of small zooplankton, we compared the TP–Chl relationship for temperate LH lakes (eq. 4) with that for subtropical SH lakes (eq. 1). This comparison of eqs. 1 and 4 showed that the TP–Chl relationship for temperate LH lakes produce a 67% lower intercept (P = 0.055) and a 22% shallower slope ($P = 0.027$; ANCOVA results, Table 2C) than subtropical SH lakes.

The greater variances of TP–Chl relationships for the temperate SH and LH lakes combined (eq. 2) than FOR subtropical SH lakes (eq. 1) may be due to the inclusion of LH lakes in the temperate data set which was not the case for the subtropical lakes. Earlier studies have shown that contrasting grazer communities can significantly influence the nutrient–phytoplankton relationships in temperate lakes (e.g., Pace 1984; Mazumder et al. 1990*b*, 1990*c*; Sarnelle 1992; Mazumder 1994*a*). Accordingly, the analysis of temperate lakes only for SH systems (Fig. 1A) produced a TP–Chl relationship even tighter than that for subtropical SH lakes. The significantly lower Chl yields for temperate LH lakes than for subtropical SH lakes (eqs. 1 and 4), but similar Chl yields between subtropical SH and temperate SH lakes (eqs. 1 and 3), demonstrate the role of grazer communities in producing different TP–Chl relationships for temperate versus subtropical lakes (Figs. 1C and 1D; Tables 2A and 2C). While several earlier papers have shown the importance of grazers in modifying Chl yields to TP in temperate lakes (Pace 1984; Sarnelle 1992;

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Table 2. ANCOVA results showing the significance of the difference in Chl yields in temperate small herbivore (SH) lakes versus subtropical SH lakes and temperate LH lakes versus subtropical SH lakes with TP and TN as covariates.

Source	SS	df	MS	*F*-ratio	*P*	r^2
(A) Temperate SH versus subtropical SH lakes						
Covariate (TP)	64	1	64	808	0.000**	0.85
Category	0.17	1	0.17	2.08	0.151 ns	
TP × category	0.11	1	0.11	1.42	0.234 ns	
Error	23	290	0.08			
(B) Temperate SH versus subtropical SH lakes						
Covariate (TN)	49	1	49	330	0.000**	0.67
Category	0.07	1	0.07	0.45	0.501 ns	
TN × category	0.01	1	0.01	0.08	0.775 ns	
Error	31	207	0.15			
(C) Temperate LH versus subtropical SH lakes						
Covariate (TP)	47	1	47	476	0.000**	0.77
Category	0.37	1	0.37	3.74	0.055*	
TP × category	0.49	1	0.49	4.96	0.027**	
Error	18	181	0.10			
(D) Temperate LH versus subtropical SH lakes						
Covariate (TN)	26	1	26	208	0.000**	0.70
Category	0.27	1	0.27	2.11	0.149 ns	
TN × category	0.34	1	0.34	2.67	0.105 ns	
Error	13	105	0.13			
(E) Comparison of third-order polynomial equations for subtropical SH and temperate SH lakes						
TP	66	1	66	839	0.000**	0.86
TP × category	0.01	1	0.01	0.09	0.763 ns	
TP^2 × category	0.05	1	0.05	0.58	0.447 ns	
TP^3 × category	0.12	1	0.12	1.56	0.213 ns	
Error	22.61	289	0.08			
(F) Comparison of third-order polynomial equations for subtropical SH and temperate LH lakes						
TP	42	1	42	419	0.000**	0.77
TP × category	1.21	1	1.21	12	0.001**	
TP^2 × category	0.45	1	0.45	4.53		0.035*
TP^3 × category	0.38	1	0.38	3.48		0.050*
Error	22.61	289	0.08			

Note: Temperate and subtropical regions were used as categories in ANCOVA analyses. Probabilities for covariate indicate the significance of slopes of TP–Chl or TN–Chl relationships. A significant *P*-value for category would suggest that Chl yields as a function TP or TN (*y*-intercept) are significantly different for the two regions. Probabilities for covariate × category interactions indicate significant differences of slopes of TP–Chl or TN–Chl relationships for the two regions. **Highly significant; *significant; ns, not significant.

Mazumder 1994*a*, 1994*b*), the results presented here are probably the first demonstration of grazer effects on TP–Chl relationships across temperate to subtropical lakes.

In addition to the similarities of the linear fits of TP–Chl relationships between temperate SH or SH and LH lakes combined and subtropical SH lakes, it was interesting to note that the subtropical SH lakes also produced sigmoidal patterns like the temperate lakes (Fig. 1B). Because LOWESS does not produce quantitative equations, we developed third-order polynomial regression equations for subtropical SH lakes and for temperate SH and LH lakes combined and separately. For subtropical SH lakes, the third-order polynomial equation was log Chl = $0.12 \pm 0.21 - 0.69 \pm 0.60(\log TP) + 1.55 \pm 0.52(\log TP^2) - 0.38 \pm 0.13(\log TP^3)$ ($r^2 = 0.83$, *F*-ratio = 81.15). For temperate SH and LH lakes combined the equation was log Chl = $0.35 \pm 0.10 - 1.07 \pm 0.25(\log TP) + 1.43 \pm 0.19(\log TP^2) - 0.30 \pm 0.04(\log TP^3)$ ($r^2 = 0.79$, *F*-ratio = 391.70). For temperate SH lakes the equation was log Chl = $0.18 \pm 0.07 - 0.15 \pm 0.21(\log TP) + 0.74 \pm 0.17(\log TP^2) - 0.14 \pm 0.04(\log TP^3)$ ($r^2 = 0.90$, *F*-ratio = 591.41). For temperate LH lakes the equation was log Chl = $0.23 \pm 0.37 - 1.11 \pm 0.80(\log TP) + 1.38 \pm 0.51(\log TP^2) - 0.29 \pm 0.10(\log TP^3)$ ($r^2 = 0.77$, *F*-ratio = 121.04). All four third-order polynomial equations produced substantial improvements in r^2 and *F*-ratios over the corresponding simple linear equations (eqs. 1–4) or corresponding second-order polynomial equations (not shown), suggesting that LOWESS and nonlinear equations provided the best fits for TP–Chl relationships for both subtropical and temperate lakes. Similar to the comparisons of linear eqs. 1 versus 3 and 1 versus 4 (Table 1), the regional comparisons of third-order polynomial equations showed that subtropical SH lakes do not produce a significantly different nonlinear equation compared

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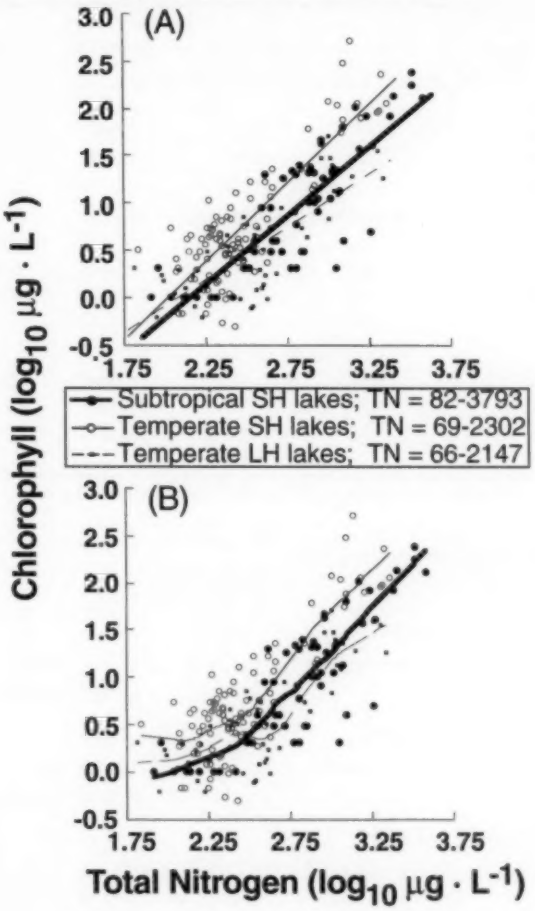
Fig. 2. Relationship between TN and Chl in temperate SH and LH and subtropical SH lakes. (A) Linear relationships; (B) LOWESS smoothing curves. Symbols and lines are the same as in Figs. 1C and 1D. The appropriate equations are presented in the text.



with temperate SH lakes, while they produce a significantly different equation compared with that for temperate LH lakes (Table 1). Although nonlinear models seemed to produce the best fits, as suggested by both LOWESS and third-order polynomial models, we based our regional comparisons of equations using linear regression equations so that our analyses are comparable with the published models on subtropical and temperate lakes.

Although several previous papers have reported sigmoidal patterns of TP–Chl relationships for temperate lakes (McCauley et al. 1989; Prairie et al. 1989; Watson et al. 1992; Mazumder 1994*a*), the only suggestion of a curvilinear pattern for subtropical lakes has been reported by Canfield (1983). However, Canfield (1983) did not demonstrate the sigmoidal patterns of TP–Chl relationships using polynomial model or model-free analysis such as LOWESS. McCauley et al. (1989) suggested that a sigmoidal pattern of the TP–Chl relationship for temperate lakes is due to the disproportionality of growth and loss rates of algae whereas Prairie et al. (1989) suggested that it may be due to the changes in the TN:TP ratio along a TP gradient. Watson et al. (1992) suggested that a sigmoidal pattern is produced by the disproportionate contribution of large inedible algae to total algal biomass with increasing TP. The pattern of larger algae with increasing trophic state has been documented for Florida lakes (Agustí et al. 1990; Duarte et al. 1990). Mazumder (1994*a*) suggested that the steeper slope of the TP–Chl relationship associated with the transition from thermally stratified to mixed lakes is a probable mechanism producing a sigmoidal pattern. In the sigmoidal relationship, the subtropical lakes seem to produce a steeper slope at a lower TP concentration (around 12 µg·L^{-1}) than the temperate lakes (around 30 µg·L^{-1}). Because we did not have the data on mixing regimes of subtropical lakes, it was not possible to test if a transition of stratified to mixed lakes was associated with the observed sigmoidal pattern.

TN–Chl and TP–TN–Chl relationships

The least square regression equations for TN–Chl relationships (Fig. 2) were as follows.

Subtropical SH lakes:

(5) $\log \text{Chl} = -3.09 \pm 0.36 + (1.44 \pm 0.13)\log \text{TN}$
$n = 59$, $r^2 = 0.69$, $P < 0.0001$.

Temperate SH lakes:

(6) $\log \text{Chl} = -3.37 \pm 0.28 + (1.66 \pm 0.11)\log \text{TN}$;
$n = 93$, $r^2 = 0.61$, $P < 0.0001$.

Temperate LH lakes:

(7) $\log \text{Chl} = -2.39 \pm 0.32 + (1.15 \pm 0.12)\log \text{TN}$;
$n = 50$, $r^2 = 0.65$, $P < 0.0001$.

Equations 5 and 6 showed that subtropical SH (eq. 5) and temperate SH (eq. 6) lakes were not significantly different either in terms of slopes ($P = 0.775$) or in terms of intercepts ($P = 0.501$) (Table 2B). However, unlike the significantly different TP–Chl relationships between subtropical SH and temperate LH lakes, the TN–Chl relationship for subtropical SH lakes (eq. 5) was similar to that for temperate LH lakes (eq. 7) ($P > 0.05$) (Table 2D). Because TN has often been suggested to be as important as TP in predicting Chl yields in lakes (Canfield 1983; McCauley et al. 1989; Prairie et al. 1989; Smith 1990), we analyzed TN–Chl relationships for subtropical SH, temperate SH, and temperate LH lakes (Fig. 2). Furthermore, the use of TN was important because it has been suggested by some that phytoplankton biomass in subtropical lakes tends to be more nitrogen limited, while in temperate lakes, biomass tends to be more phosphorus limited (Henry et al. 1985; Dierberg et al. 1988; Davalos et al. 1989; Hecky et al. 1993). However, only a subset of the temperate lakes had data for TP as well as TN, and therefore, we derived TN–Chl relationships using the smaller subset of temperate SH lakes ($n = 93$) and the complete data set for the subtropical lakes (Fig. 2). Like the similar TP–Chl relationships for subtropical and temperate SH lakes (Table 2), the TN–Chl relationship for temperate SH lakes does not seem to be different from that for the subtropical SH lakes (Fig. 2A).

Although the LOWESS fits to TN–Chl data (Fig. 2B) indicate that the Chl yield per unit TN is higher for temperate SH

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lakes across the entire gradient of TN, the regional comparisons of subtropical SH versus temperate SH or LH lakes did not indicate significantly different second- or third-order polynomial equations (ANCOVA, P ranged from 0.657 to 0.960). Neither for subtropical nor for temperate lakes did TN–Chl relationships exhibit sigmoidal patterns, although Canfield (1983) suggested a nonlinear TN–Chl relationship for subtropical lakes. If subtropical lakes were more nitrogen limited than temperate lakes, we should have observed an elevated Chl yield per unit TN for subtropical SH lakes as compared with temperate SH lakes. Instead, Chl yields to TN are higher, although not significant, for temperate SH lakes than for subtropical SH lakes. One explanation that has been proposed is that, in subtropical lakes, a larger portion of the TN pool is associated with humic material unavailable to phytoplankton (Walker and Havens 1995). Similar TN:TP ratios (Table 1; ANOVA, $P = 0.814$) do not suggest any different role of nitrogen versus phosphorus for subtropical SH and temperate SH lakes. The observed TN:TP ≈ 40 would suggest phosphorus rather than nitrogen limitation of algal biomass development in both types of lakes. However, it is not clear why the temperate LH lakes have a significantly lower TN:TP ratio (16) than either temperate SH or subtropical SH lakes (ANOVA, $P = 0.000$).

The multivariate TP–TN–Chl equations were as follows.

Subtropical SH lakes:

(8) $\log \text{Chl} = -1.39 \pm 0.41 + (0.78 \pm 0.13)\log \text{TP} + (0.49 \pm 0.19)\log \text{TN}$; $n = 59$, $R^2 = 0.81$, $P < 0.0001$.

Temperate SH lakes:

(9) $\log \text{Chl} = -1.99 \pm 0.28 + (0.51 \pm 0.07)\log \text{TP} + (0.90 \pm 0.13)\log \text{TN}$; $n = 93$, $R^2 = 0.82$, $P < 0.0001$.

The multivariate regression equations with TP and TN as the two variables predicting Chl showed that the addition of TN to TP–Chl relationships produces significant improvements over TP–Chl and TN–Chl relationships (partial P for TP and TN < 0.001, Table 3). The analyses of TP–Chl and TN–Chl and multivariate analyses suggest that phytoplankton in both subtropical and temperate lakes responds significantly to TN as a second independent variable. Canfield (1983) made a similar suggestion based on comparisons of published empirical equations on temperate lakes (Dillon and Rigler 1974; Jones and Bachmann 1976) with data he collected on subtropical lakes. However, it seems that the addition of TN to TP–Chl relationships has a more significant influence in predicting Chl in temperate SH lakes (ANOVA for eq. 9, F-ratio = 315, $P < 0.001$) than in subtropical SH lakes (eq. 8, F-ratio = 126, $P = 0.012$) (Table 3). Furthermore, it is notable that for subtropical SH lakes the slope for TP is greater than the slope for TN, while for temperate SH lakes the slope for TP is almost half the slope for TN. A greater slope for TN for subtropical SH lakes than for temperate SH lakes should have been observed to support the hypothesis that subtropical lakes are more nitrogen limited than temperate lakes. The ANOVA results of multivariate analyses show that significant impact of TN as a second independent variable in predicting Chl is also observed for temperate LH lakes.

Table 3. ANOVA results of univariate and multivariate least square regressions showing significance of TP and TN in explaining variance in Chl in temperate small herbivore (SH) and large herbivore (LH) lakes and subtropical SH lakes.

Independent variable	F-ratio	Partial P	Overall P	R^2
(A) Temperate SH lakes ($n = 93$)				
TP	456		0.000	0.75
TN	286		0.000	0.66
TP +	315	0.000	0.000	0.82
TN		0.000		
(B) Subtropical SH lakes ($n = 59$)				
TP	210		0.000	0.79
TN	207		0.000	0.69
TP +	126	0.000	0.000	0.81
TN		0.012		
(C) Temperate LH lakes ($n = 50$)				
TP	92		0.000	0.66
TN	87		0.000	0.65
TP +	66	0.000	0.000	0.74
TN		0.000		

Note: Only a subset of the lakes was used for these analyses (see text).

Transparency relationships

The Chl–Secchi equations were as follows.

Subtropical SH lakes:

(10) $\log \text{Secchi} = 0.59 \pm 0.03 - (0.43 \pm 0.04)\log \text{Chl}$; $n = 59$, $r^2 = 0.76$, $P < 0.001$.

Temperate SH lakes:

(11) $\log \text{Secchi} = 0.74 \pm 0.04 - (0.46 \pm 0.03)\log \text{Chl}$; $n = 85$, $r^2 = 0.77$, $P < 0.001$.

Temperate LH lakes:

(12) $\log \text{Secchi} = 0.85 \pm 0.09 - (0.42 \pm 0.04)\log \text{Chl}$; $n = 40$, $r^2 = 0.77$, $P < 0.001$.

Comparison of eqs. 10 and 11 showed that temperate SH lakes produce greater water transparency or deeper Secchi depth per unit Chl than subtropical SH lakes (Fig. 3A; Table 4A, ANCOVA, P for category = 0.000). Similarly, the temperate LH lakes (eq. 12) showed even deeper Secchi depth as a function of Chl than subtropical SH lakes (Table 4B, P for category = 0.000). However, a shallower slope of the Chl–Secchi relationship for temperate SH lakes than for subtropical SH lakes (P for Chl × category = 0.002) suggests that Secchi depth is not only shallower, but also declines less as a function of Chl in subtropical SH lakes than in temperate SH lakes. Based on eqs. 10–12, a Chl concentration of 5 $\mu g \cdot L^{-1}$ predicts average Secchi depths of 1.9, 2.6, and 3.6 m for subtropical SH, temperate SH, and temperate LH lakes, respectively.

The least square regression equations for TP–Secchi relationships were as follows.

Subtropical SH lakes:

(13) $\log \text{Secchi} = 0.86 \pm 0.05 - (0.55 \pm 0.04)\log \text{TP}$; $n = 59$, $r^2 = 0.78$, $P < 0.001$.

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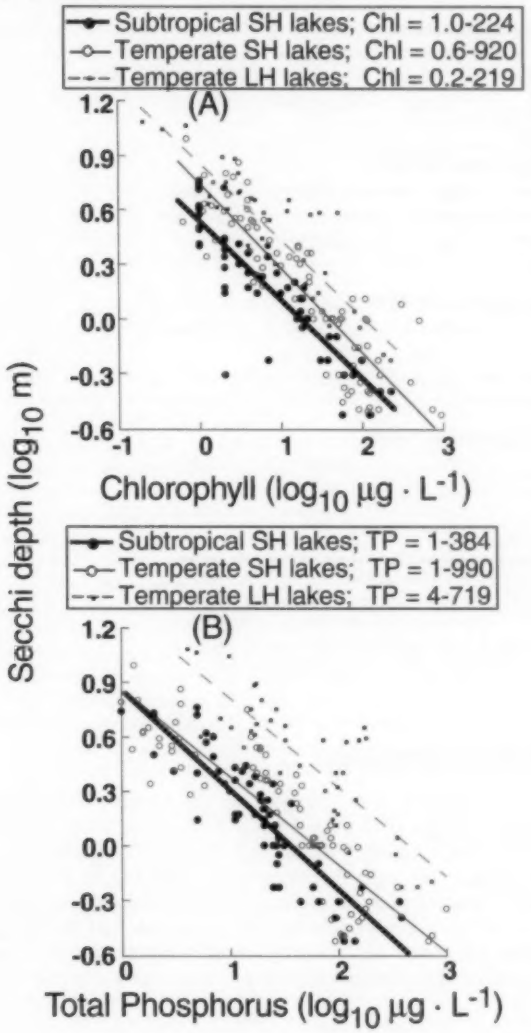
Fig. 3. Relationship between (A) Chl and Secchi depth and (B) TP and Secchi depth for temperate SH and LH and subtropical SH lakes. Symbols and lines are the same as in Figs. 1C and 1D. The appropriate equations are presented in the text.


Subtropical SH lakes; Chl = 1.0-224
Temperate SH lakes; Chl = 0.6-920
Temperate LH lakes; Chl = 0.2-219
(A)
Secchi depth (log10 m)
Chlorophyll (log10 µg · L-1)
Subtropical SH lakes; TP = 1-384
Temperate SH lakes; TP = 1-990
Temperate LH lakes; TP = 4-719
(B)
Total Phosphorus (log10 µg · L-1)

Temperate SH lakes:

(14) log Secchi = 0.86 ± 0.05 – (0.48 ± 0.03)log TP;
$n = 85$, $r^2 = 0.75$, $P < 0.001$.

Temperate LH lakes:

(15) log Secchi = 1.31 ± 0.09 – (0.49 ± 0.06)log TP;
$n = 40$, $r^2 = 0.69$, $P < 0.001$.

The TP–Secchi relationship for subtropical SH lakes (eq. 13) produced exactly the same *y*-intercept as the temperate SH lakes (eq. 14) (Fig. 3B) (ANCOVA, P for category = 0.572), but the slope was steeper for subtropical SH lakes (ANCOVA, P for TP × category = 0.041). The comparison of TP–Secchi relationships for subtropical SH (eq. 14) and temperate LH (eq. 15) lakes exhibited significantly shallower Secchi depth (P for category = 0.000) as well as faster declines in Secchi depth (P for TP × category = 0.000) as a function of TP (Table 4D). Based on eqs. 13–15, a TP concentration of 10 $\mu g \cdot L^{-1}$ predicts Secchi depths of 2.04, 2.39, and 6.60 m in subtropical SH, temperate SH, and temperate LH lakes, respectively.

A potential mechanism producing a deeper Secchi depth for a given Chl concentration in temperate SH or LH lakes than in subtropical SH lakes may be due to a larger mean size of algae in temperate lakes. Earlier literature has suggested that large filamentous algae are dominant (Crisman 1990; Crisman et al. 1995) and that the mean size of algae increases with biomass among subtropical lakes (Agustí et al. 1990; Duarte et al. 1990). A shallower slope for the Chl–Secchi relationship for subtropical SH lakes compared with that for temperate SH lakes would suggest increasing algal size with increasing biomass. We do not know if the increase in mean algal size with Chl is greater for subtropical SH lakes than for temperate SH lakes. However, if the overall mean size of algae were larger in subtropical lakes than in temperate lakes, then we should have observed deeper Secchi depth per unit Chl. It is also possible to have a shallower Secchi depth per unit Chl if Secchi depth is attenuated also by something other than algae, for example, by inorganic clay particles and dissolved humics. We could not test this phenomenon in this paper because such information was not available. A deeper Secchi transparency with increasing mean size of plankton has been shown for temperate lakes (Mazumder et al. 1990*a*, 1990*b*). The temperate LH lakes, which can be assumed to have even larger mean size of algae, produce significantly deeper Secchi depth than either subtropical SH or temperate SH lakes (Fig. 3; Table 4).

It is notable that while the slope of the Chl–Secchi relationship is steeper for temperate SH compared with subtropical SH lakes, the slope of the TP–Secchi relationship is steeper for subtropical SH lakes compared with temperate SH lakes. The potential mechanisms for such a reversal in slopes of Chl–Secchi and TP–Secchi from subtropical SH to temperate SH lakes are not entirely clear. Is it possible that algal mean size as a function of biomass declines faster in temperate SH lakes than in subtropical SH lakes, while the overall mean size is smaller in subtropical SH lakes. On the other hand, a steeper TP–Secchi relationship for subtropical SH lakes compared with temperate SH lakes would suggest the contrary unless other factors like clay particles and dissolved humics are responsible. A faster decline in Secchi depth as a function of TP than as a function of Chl in subtropical SH than in temperate SH lakes could also be due to a relatively greater contribution of clay particles in attenuating water transparency in high-TP subtropical lakes. In temperate lakes, algal size has been suggested to increase with increasing TP (Watson et al. 1992), especially under the dominance of large grazers (Mazumder et al. 1988; Mazumder 1994*b*). Other experiments have shown that mean size of algae decreases with increasing TP or Chl when small zooplankton are the dominant grazers (Larocque et al. 1996). A given concentration of Chl should produce shallower Secchi depth under smaller mean size of algae. Due to lack of data on the relative contribution of Chl and the size distribution of algae and of clay particles, we could not test our hypothesis. Further research would be needed to address this concept.

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Table 4. ANCOVA results showing the significance of the differences in Secchi depth in temperate small herbivore (SH) lakes versus subtropical SH lakes and temperate large herbivore (LH) lakes versus subtropical SH lakes as a function of Chl and TP.

Source	SS	df	MS	*F*-ratio	Partial *P*	r^2
(A) Temperate SH versus subtropical SH lakes						
Covariate (Chl)	9	1	9	111	0.000**	0.47
Category	0.38	1	0.38	4.69	0.032*	
Chl × category	0.78	1	0.78	9.78	0.002**	
Error	10	128	0.08			
(B) Temperate LH versus subtropical SH lakes						
Covariate (Chl)	23	1	23	298	0.000**	0.86
Category	14	1	15	203	0.000**	
Chl × category	2.94	1	2.95	38	0.000**	
Error	7	95	0.08			
(C) Temperate SH versus subtropical SH lakes						
Covariate (TP)	10	1	10	133	0.000**	0.51
Category	0.02	1	0.02	0.32	0.572 ns	
TP × category	0.31	1	0.31	4.26	0.041*	
Error	9	128	0.07			
(D) Temperate LH versus subtropical SH lakes						
Covariate (TP)	21	1	21	224	0.000**	0.82
Category	14	1	14	142	0.000**	
TP × category	2.56	1	2.56	27	0.000**	
Error	9	95	0.09			

Note: Temperate and subtropical regions were used as categories in ANCOVA analyses. Probabilities for covariate indicate the significance of slopes of Chl–Secchi or TP–Secchi relationships. A significant *P*-value for category would suggest that Chl yields as a function Chl or TP (*y*-intercept) are significantly different for the two regions. Probabilities for covariate × category interactions indicate significant differences of slopes of Chl–Secchi or TP–Secchi relationships for the two regions. **Highly significant; *significant; ns, not significant.

Conclusions

(*i*) The TP–Chl relationships for subtropical SH and temperate SH lakes are similar; both produce significantly higher Chl per unit TP than temperate LH lakes. TP–Chl relationships are sigmoidal for all three types of lakes.

(*ii*) Nitrogen does not explain a greater portion of Chl variance in subtropical lakes compared with temperate lakes. Both subtropical SH and temperate LH lakes seem to be phosphorus limited.

(*iii*) Secchi transparency at a given Chl or TP concentration decreases from temperate LH to temperate SH to subtropical SH lakes. Although the rate of decline in Secchi transparency as a function Chl and TP seems to reverse significantly from temperate SH to subtropical SH lakes, the differences are not too large.

(*iv*) The overall analyses of nutrient–Chl–transparency relationships suggest a potentially important role of zooplankton grazers in determining Chl and water transparency in these two distinctly different geographical zones. We could not test if LH-dominated systems would produce different Chl or water clarity responses to nutrients in temperate versus subtropical lakes because large herbivorous zooplankton were not present in the subtropical lakes used in this study. However, the recent invasion of subtropical lakes by the large-bodied tropical *Daphnia lumholtzii* (Havel and Hebert 1993; Havens and East 1997) may change this situation in the future.

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Otolith and fish size relationships, measurement error, and size-selective mortality during the early life of Atlantic salmon (*Salmo salar*)[1]

M.G. Meekan, J.J. Dodson, S.P. Good, and D.A.J. Ryan

Abstract: The development of the relationship between otolith and body size in Atlantic salmon (*Salmo salar*) between hatching and emergence was examined by repeatedly measuring individually identified fish. Otolith growth increments were deposited daily in the period between hatching and emergence. Comparison of back-calculated otolith size and standard length using least squares regression analyses revealed a weak relationship between these variables at each of the 5-day sampling intervals. However, when data sets were pooled among intervals, variation in otolith size accounted for 98% of the variation in alevin length. A computer simulation demonstrated that levels of measurement error similar to those documented in our study resulted in the failure of regression analyses to detect strong relationships between otolith and fish size. Mortality that occurred during the experiment was strongly size selective. This truncated the size ranges of fish in cross-sectional data sets and thus reduced the ability of regression analysis to detect relationships between otolith and fish size. We propose that the weak relationship between otolith and fish size at emergence recorded in previous studies was an artifact of measurement error and the truncation of size ranges in regression analyses. Differences in alevin size at emergence were present at hatching and had been propagated by growth.

Résumé : Nous avons examiné le développement du rapport entre la taille des otolithes et la taille corporelle chez le saumon atlantique (*Salmo salar*), entre l'éclosion et l'émergence, en mesurant à plusieurs reprises chacun des poissons marqués. Au cours de cette période, les cercles de croissance se sont déposés tous les jours sur les otolithes. La comparaison de la taille des otolithes et de la longueur standard obtenues par rétrocalcul à l'aide des analyses de régression des moindres carrés a révélé un faible rapport entre ces variables pour chacun des intervalles d'échantillonnage de 5 jours. Toutefois, lorsque les ensembles de données ont été regroupées entre intervalles, la variation de la taille des otolithes représentait 98% de la variation de la longueur des alevins. Une simulation sur ordinateur a montré que des taux d'erreur de mesure semblables à ceux de notre étude étaient attribuables au fait que les analyses de régression ne permettaient pas de déceler de relations fortes entre la taille des otolithes et la taille du poisson. La mortalité enregistrée pendant l'expérience était fortement sélective selon la taille, ce qui a tronqué les plages de taille des poissons dans les ensembles de données transversales, et a donc réduit la capacité de l'analyse de régression à déceler les rapports entre la taille des otolithes et la taille du poisson. Selon nous, le faible rapport entre la taille des otolithes et la taille du poisson au moment de l'émergence enregistré dans des études précédentes était un artefact de l'erreur de mesure et de la troncature des plages de taille dans les analyses de régression. Les différences de taille chez les alevins à l'émergence étaient présentes à l'éclosion et elles se sont maintenues tout au long de la croissance.

[Traduit par la Rédaction]

Introduction

The analysis of age and growth information contained in the otoliths of young fish has become a standard technique in fisheries science. Typically, growth increments are deposited within otoliths on a daily basis and thus provide an estimate of an individual's age (Jones 1992). In many species, the timing of major life history events such as hatching or the initiation of first feeding can also be determined, since these may coincide with the deposition of distinctive increments within the otolith (Victor 1982). Furthermore, past histories of growth may be reconstructed from the relative spacing between growth increments (Campana 1990).

The reconstruction of growth patterns from otoliths relies on two assumptions. Firstly, deposition rates of increments must be regular and invulnerable to systematic errors when the otolith is analyzed. Secondly, the back-calculation of growth rates assumes that there is an allometric relationship between

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growth of the otolith and somatic growth of the fish. In recent years, a large number of studies have drawn attention to the problems of error and bias associated with these assumptions (Campana 1992; Neilson 1992). In a few cases, workers have reported that increments within the otolith were not formed on a daily basis (Geffen 1982; Campana 1984), while other studies have shown that the relationship between otolith and somatic growth may be lagged (Molony and Choat 1990) or very weak (Mosegaard et al. 1988; Secor and Dean 1989; Wright et al. 1990). When such problems occur, otoliths will be of limited use in the back-calculation of growth rates.

In the weeks after emergence, strong selection occurs in Atlantic salmon (*Salmo salar*) and other salmonids, with the larger, faster growing members of the cohort occupying and defending the most profitable territories in the streambed (Elliot 1990). The smaller, slower growing fish are forced downstream and may eventually die. Among those that survive, relative dominance status and competitive ability determine subsequent life history patterns (Metcalfe et al. 1990; Metcalfe 1991). Individuals that grow quickly may migrate to the sea after only 1 year in streams, while slow-growing fish delay migration, remaining in the stream for at least a second year (Thorpe 1977, 1989; Wright et al. 1990). The probability of survivorship and the establishment of a territory is correlated with otolith size, so that fast-growing, dominant individuals generally have larger otoliths at emergence than the slow-growing members of the same cohort (Metcalfe et al. 1992). This implies that the process of selection must begin at the initiation of first feeding.

As salmon otoliths are formed in the developing embryos prior to hatching (Geffen 1983), the records of growth retained in these structures may provide a means of examining the growth rates and size of fish during the earliest stages of the life history. However, Geffen (1983) found that during the period between hatching and exogenous feeding when the alevins reside in the redd, otolith growth increments may not be deposited at daily intervals. Furthermore, there appears to be little relationship between otolith and fish size at the time of emergence (Metcalfe et al. 1992). These problems seriously impair efforts to reconstruct growth histories from the otoliths of young salmon.

The lack of correlation between otolith and fish size at emergence and first feeding stands in marked contrast with patterns found only a few weeks later in the life history, when deposition rates within the otolith follow a regular daily pattern and there is a good concordance between otolith and fish size (Titus and Mosegaard 1991; Wright et al. 1991; Metcalfe et al. 1992). To account for this apparent contradiction, some workers have attempted to explain the convergence of otolith and fish growth rates as a function of the physiological process of otolith formation in salmon. This argument proposes that during the period prior to and immediately following emergence, variation in otolith size reflects differences in metabolic rate among individuals, so that fish with high metabolic rates tend to have larger otoliths, but are not necessarily larger in size (Mosegaard 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992). As individuals with higher metabolic rates grow faster, otolith size and fish size become correlated once relatively large size differences develop after a few weeks of life in the stream habitat.

There are, however, alternative explanations of changes in the strength of the relationship between otolith and fish size in young salmon. At emergence, variability in alevin size is small. This restriction of size ranges has important implications, since comparisons of otolith and fish size are typically made using least squares regression methods that are vulnerable to sample truncation effects (Campana 1990; Campana and Jones 1992; Murdoch and Doherty 1997). Furthermore, it will be difficult to detect real differences among individuals if errors in measuring small otoliths and alevins are relatively large (Meekan 1997). Since variation in growth rates among alevins will have cumulative effects on relative size, fry collected some weeks or months after emergence will display a much greater range of sizes at any given age. In contrast, measurement errors should remain constant and will be proportionally smaller, relative to size differences among individuals. Consequently, any relationship between otolith and fish size will be easier to detect using regression analysis. Thus, variation in size at age and the relative importance of measurement error may account for the lack of correlation between otolith and fish size early in the life of Atlantic salmon.

Here, we examine the extent to which otoliths provide an accurate estimate of age and growth of Atlantic salmon during the period between hatching and emergence. The accuracy of age estimates was determined by comparing counts of increments within otoliths with the known age of individuals. The development of otolith and fish size relationships was examined by repeatedly measuring individually identified fish in the period between hatching and emergence. Relationships between otolith and fish size in these fish were compared with those of alevins collected at hatching and emergence.

Methods and materials

Experimental design and sampling

Embryos were obtained from sea-run salmon caught in the Sainte-Marguerite River, Québec, Canada, in the fall of 1994. These were placed in a flow-through incubator that drew water directly from a tributary branch of the river. Within the incubator, eggs were spread in thin layers on perforated racks. At hatching, which occurs in the early spring, alevins migrate into gravel at the bottom of the incubator. After about 20–25 days, yolk reserves are exhausted, and alevins emerge from the gravel to be carried by the current to the outflow pipe. Alevins are then netted and released into the river.

On May 18, 1995, a few days before hatching, 100 embryos were placed in a fine-mesh cage within the incubator and examined daily for hatching. Alevins were removed on the day of hatching, filmed using a video camera, and then frozen. A sample of 100 emergent alevins that were collected on the first day of their appearance at the outflow pipe of the incubator was also filmed and preserved in liquid nitrogen. In addition to these regular samples of the population held within the incubator, the individual growth rates of 200 alevins were recorded. These individuals were haphazardly selected just before hatching, placed in individually numbered containers in the incubator, and monitored daily for hatching. At hatching, containers were removed and the alevins filmed. Containers were then replaced in the incubator. This process was repeated at 5-day intervals for 20 days, at which time the surviving alevins were removed, filmed, and preserved in alcohol. This gave a total of five repeated measures of these individuals. The final days of this experiment coincided with the emergence of the majority of alevins from the gravel in the incubator. During the period of the experiment, alevins in the incubator were exposed to natural cycles of light and water temperature. Average water temperature in the incubator and in the stream that supplied the

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flow-through system was 10°C (SD = 3.6) and ranged from 5.0°C at the beginning of the experiment to 15.5°C at the end.

Otolith and size analysis

Sagittae and lapilli were dissected from the alevins using fine forceps and a dissecting microscope with a polarized light source. They were cleaned and placed in thermoplastic glue on a microscope slide. Otoliths from the larger individuals (those near to or at emergence) were ground on lapping film to the level of the core of the otolith. Preparations were viewed using a compound microscope at magnifications of 250 and 400× that was linked by a high-resolution video camera to a video monitor and computer. Increments were counted and measured along a constant axis in one of the pair of sagittae (the largest of the pairs of otoliths) with the aid of an image analysis program (OPTIMAS). Total area of the otolith was also measured. Similarly, standard lengths and area of the yolk sac of alevins were obtained from the videotape records.

Data analysis

Otolith – somatic growth relationships

Standard lengths and otolith dimensions were compared using regression analysis. However, for individuals that were repeatedly measured, this analysis could not be used on the entire data set of length and otolith measurements (the five sampling intervals), since data points collected at different times were not independent. The complete data set was analyzed by fitting the following random coefficient model to the data using restricted maximum likelihood techniques (PROC MIXED, SAS V6):

$$y_{ij} = \rho + \delta_{\rho_i} + (\beta_1 + \delta_{\beta_{1i}})x_{ij} + (\beta_2 + \delta_{\beta_{2i}})x_{ij}^2 + \varepsilon_{ij}$$

where y_{ij} is the fork length and x_{ij} is the otolith radius for the ith individual at the jth time period, ρ, β_1, and β_2 represent the intercept, slope, and quadratic terms, respectively, and δ_{ρ_i}, $\delta_{\beta_{1i}}$, and $\delta_{\beta_{2i}}$ are independent random deviations from the intercept, slope, and quadratic terms and are assumed to be distributed as $NI(0, \sigma_\rho^2)$, $NI(0, \sigma_{\beta_1}^2)$, and $NI(0, \sigma_{\beta_2}^2)$, respectively. The error term, ε_{ij} was assumed to be distributed as $N(0, \sigma_{ij}^2)$ and $Cov = (\varepsilon_{ij}, \varepsilon_{i^*j}) = 0$ for $i \neq i^*$ and $Cov = (\varepsilon_{ij}, \varepsilon_{i^*j}) = \sigma_{ij^*}$ for $i = i^*$. This covariance structure was used to account for the correlations between measurements made on the same individual through time (Laird and Ware 1982; Chambers and Miller 1995).

Measurement error

To quantify the error involved in measurements of otolith dimensions, the radius and area of the otoliths of 10 haphazardly selected alevins were remeasured five times. The error generated from measurements of standard length was estimated by reanalyzing all videotapes taken during the course of the experiment, 2 months after they were initially analyzed.

The influence of measurement errors on the ability of regression analyses to detect relationships between otolith and fish size at emergence was examined in a computer simulation. Otolith radius was used to generate predicted standard length using the regression relationship calculated for fish at the end of the 20-day experiment. It was assumed that a perfect correlation existed between these variables (i.e., $r^2 = 1$). The effect of measurement error was evaluated by adding a random error component to observed otolith radii and predicted standard lengths using the RANUNI function of SAS (v.6). This error component had a mean of 0 and a standard deviation that was chosen to correspond to the size of the errors estimated from the repeated measurements of the otolith radii and standard lengths of the same individuals. A total of 500 data sets were generated, each containing 97 paired observations of otolith radius and standard length. Regression analyses were then used to estimate the mean r^2 and its standard deviation from the 500 data sets. The standard deviation of the error component added to the observed otolith radius and predicted standard lengths was then reduced and the entire simulation repeated. The influence of differing measures of otolith size on regression analyses was examined by repeating the process outlined above using otolith area (with appropriate regression model and error components) rather than radius in the simulation.

Size-selective survivorship

We examined the influence of selective mortality on survivorship during our experiment by comparing frequency distributions of standard length at hatching of individuals that survived the 20-day period of the experiment with those of individuals that had died 5, 10, and 15 days after the experiment began. As the death of an alevin did not always coincide with a day during the experiment when standard lengths were measured, dead individuals were discarded after they were removed from containers held in the incubator. Consequently, frequency distributions of otolith size of individuals that survived could not be compared with those of alevins that died. However, we were able to compare the growth of survivors with that of dead alevins by reconstructing growth trajectories from videotape records taken in the time periods prior to their death. As samples sizes at each time interval (i.e., 5, 10, 15, and 20 days) were unequal due to mortality, these repeated measures data sets were analyzed as a mixed linear model (Laird and Ware 1982; Chambers and Miller 1995) using PROC MIXED, SAS (v.6).

Results

Age

The sagittae and lapillae of alevins that had been placed in individual containers at hatching and then killed after 20 days had an average of 23.5 (SD = 1.3, range 17–27) and 22.68 (SD = 1.5, range 17–25) growth increments, respectively. In all individuals, there was a distinctive mark near the centre of the otolith that was formed about 20 increments before the end of the experiment (Fig. 1). As this mark was also present at the margin of otoliths of newly hatched alevins, it was probably formed just prior to or on the day of hatching. In the region of the otolith within the hatchmark, two or three faint increments were visible (Fig. 2), which must have been deposited prior to hatching. Thus, the rate of increment deposition in otoliths of alevins was daily between hatching and emergence.

The density of growth increments, as visually assessed, was markedly reduced during the last 2 or 3 days of daily deposition in otoliths of individuals that had completely absorbed yolk reserves before the end of the experiment (Fig. 1). This transition was also found in the otoliths of all alevins that were collected from the incubator at emergence. In most individuals, this transition usually occurred two or three increments prior to the edge of the otolith (Fig. 3). These additional increments on the edge of the otolith most probably represent the number of days between yolk sac absorption and emergence from the gravel and arrival at the outflow pipe for collection.

Variation in size and growth rate

Of the 200 embryos that were placed in individual containers, 111 survived to 20 days after hatching. The standard lengths of these alevins (15.75 mm, SD = 0.52) were not significantly different from those of the population within the incubator at the start of the experiment (15.77 mm, SD = 0.44) (one-way ANOVA, $p > 0.05$). However, at 20 days after hatching, the mean size of alevins held in individual containers

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Fig. 1. Sagittal otolith of an alevin sacrificed 20 days after hatching and grown in an individual container. N, nucleus; H, hatch mark; YA, yolk sac absorption mark. The straight white line indicates the radius of the otolith, and the white flags indicate 20 daily growth increments. Yolk sac absorption occurred 17 days after hatching. Also note the presence of growth increments prior to the hatch mark.

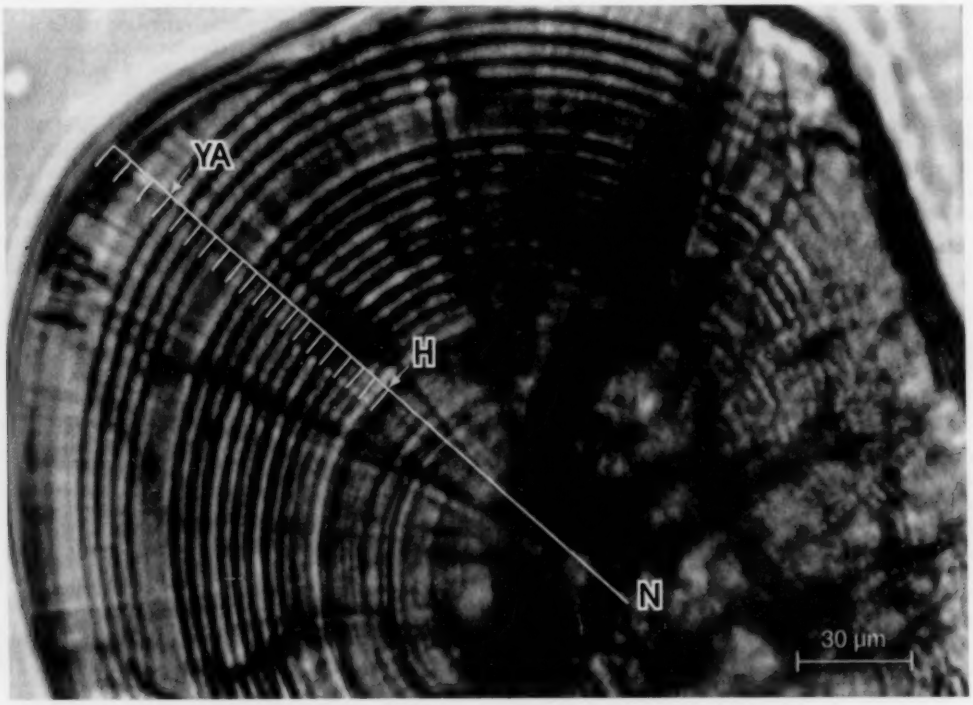

Fig. 2. Sagittal otolith of an alevin taken on the day of hatching. N, nucleus; H, hatch mark. The straight black line indicates the radius of the otolith, and the black flags identify five increments deposited prior to the hatch mark.

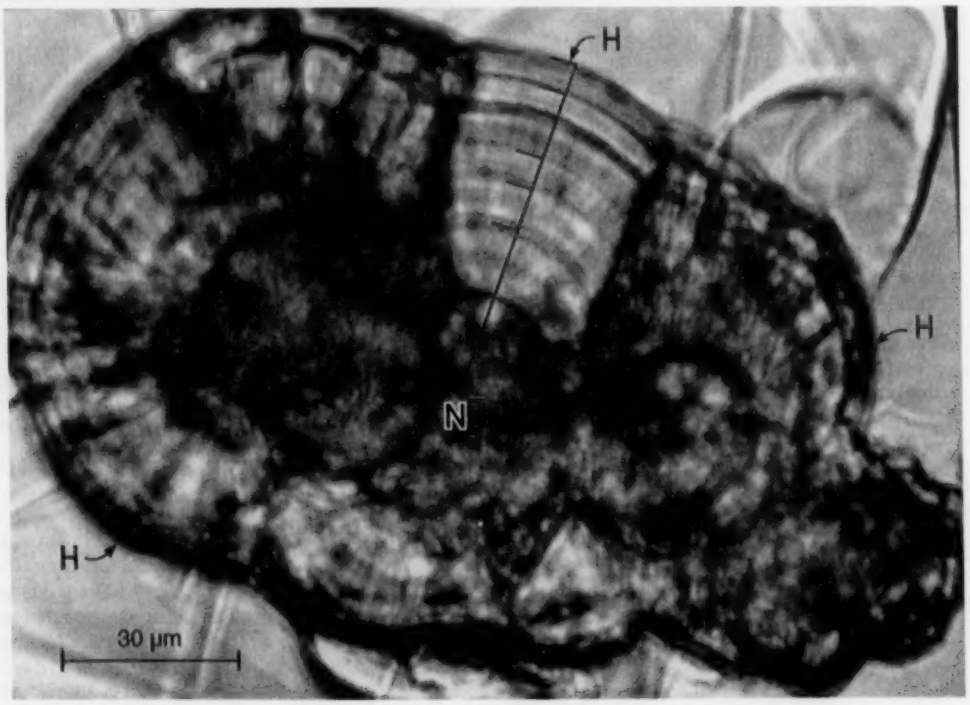

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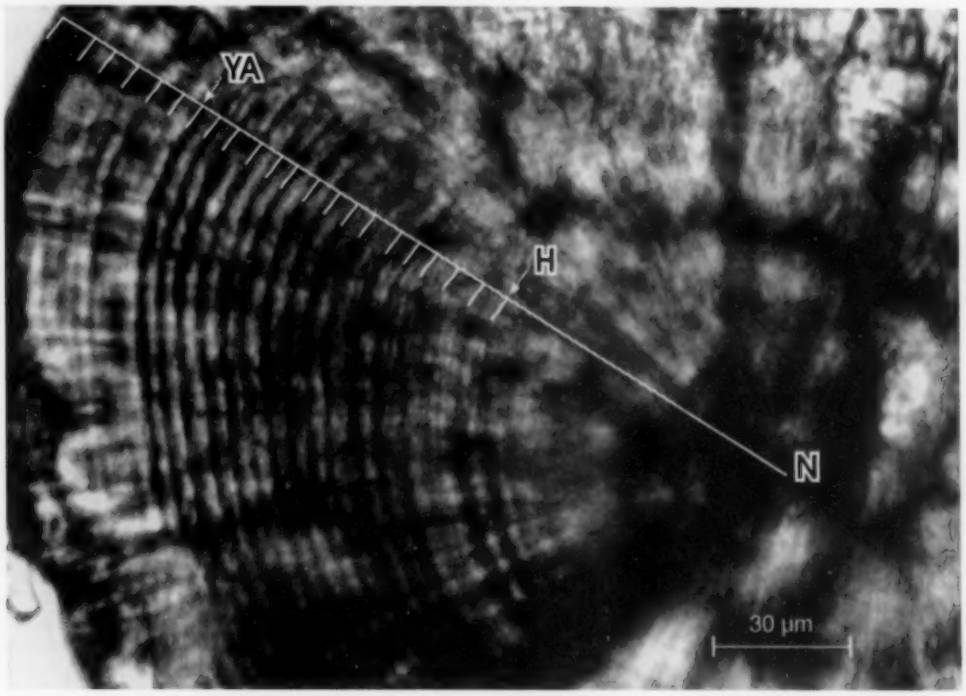
Fig. 3. Sagittal otolith of an alevin taken on emergence in the first compartment of the incubator. N, nucleus; H, hatch mark; YA, yolk sac absorption mark. The straight white line indicates the radius of the otolith, and the white flags indicate daily increments. This alevin absorbed its yolk sac 16 days after hatching and emerged from the gravel 6 days later.



(23.50 mm, SD = 0.71) was smaller than that of alevins that emerged from the incubator (24.95 mm, SD = 1.10) (one-way ANOVA, $p < 0.001$). Alevins held in individual containers grew at rates of 0.57 mm·day^{-1}.

Otolith and fish size

As increments found within the otoliths of alevins were deposited at daily intervals, the relationship between otolith size and standard length could be examined for alevins that were maintained in individual containers by back-calculating the radius of the otolith on the days when measurements of size were made. Of the 111 alevins that survived until the end of the experiment, 97 were available for otolith analysis. Fourteen alevins were excluded, as clear images could not be obtained from videotapes or otoliths were lost or broken during dissection and polishing.

Significant but weak linear relationships were found between the back-calculated radius of the sagitta and standard length of the alevins (obtained from videotape images) on each of the five sampling occasions (Figs. 4A–4E). At best, variation in the radius of the sagitta could account for only 17% of the variance in fish size. There was some improvement in the strength of relationships between otolith and fish size when otolith area was used as a measure of otolith size. When total areas of sagittae were compared with the standard length of alevins collected at hatching and at the end of the experiment, there was a marked increase in the r^2 values of the relationship between otolith and fish size, with variation in the total area of the sagittae accounting for 39 and 44% of the variation in standard length at hatching and after 20 days, respectively.

When a linear model (PROC MIXED) was used to describe the relationship between the entire data set of standard length and otolith radii measurements (the five sampling intervals), these variables were highly correlated, with variation in otolith size explaining 95% of the variance in fish size. The addition of a quadratic term explained a small, but significant, amount of variability ($p < 0.0001$), with variation in otolith size explaining 98% of the variation in standard length. Thus, in contrast with the regression analyses conducted on cross-sectional data sets from each time interval, the analysis showed that when pooled among sampling times, these variables were highly correlated, with variation in otolith size explaining almost all of the variation in standard length of alevins (Fig. 4F). The covariance parameter estimates for the intercept ($\sigma^2_{\beta_0}$), slope ($\sigma^2_{\beta_1}$), and quadratic ($\sigma^2_{\beta_2}$) components were 0.4266, 0, and 0, respectively. This suggested that while fish hatched at different sizes, trajectories of fish and otolith growth were parallel among individuals. To confirm this result, the relative sizes of otolith radii of alevins at hatching were compared with their radii at emergence. Alevins were equally divided among three categories (small, medium, and large) on the basis of otolith radius firstly at hatching and then again at the end of the experiment. The composition of these categories was compared using a chi-square test (Table 1). This analysis was then repeated using standard lengths of alevins at hatching and emergence (Table 1). As in the results of the modeling of otolith and fish size, these comparisons showed that the

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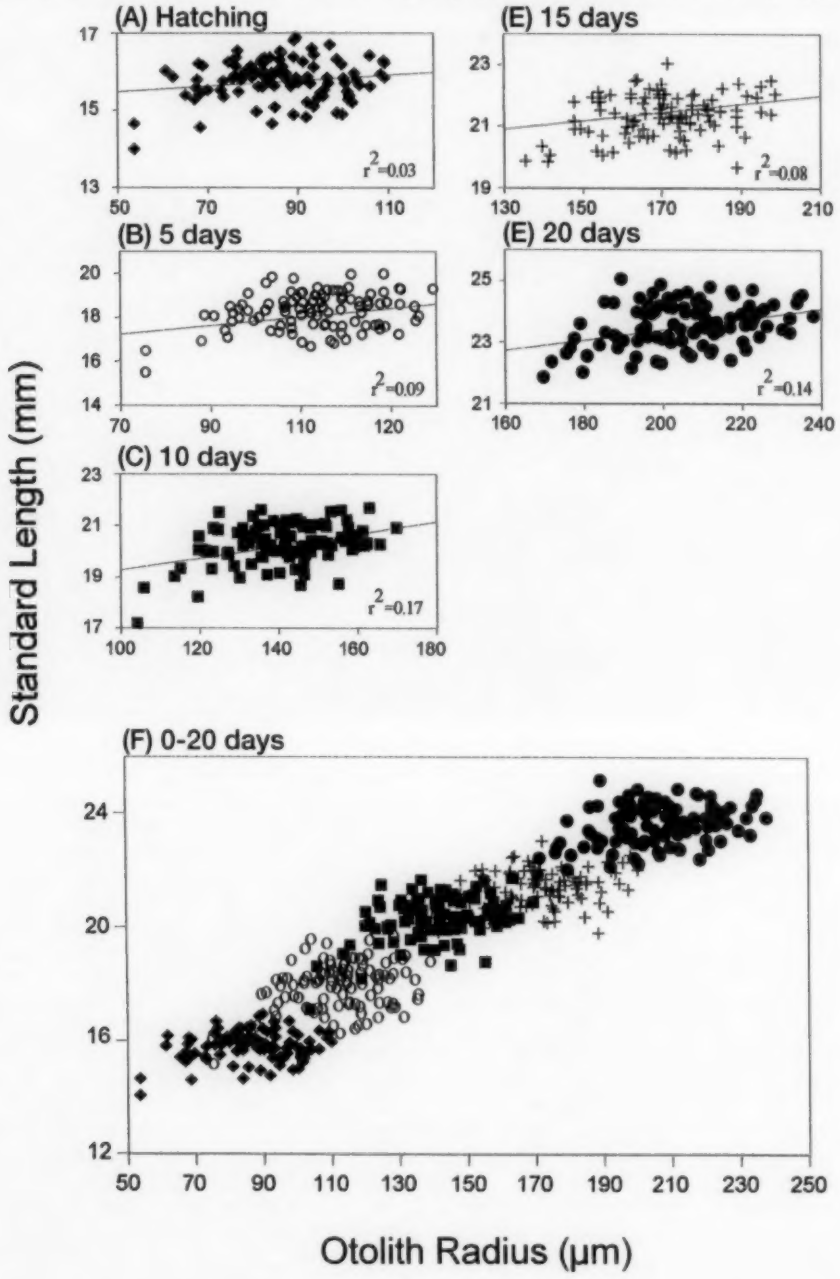
Fig. 4. Relationships between otolith radius and standard length of salmon larvae sampled at (A) hatching and (B) 5 days, (C) 10 days, (D) 15 days, and (E) 20 days after hatching; (F) data sets pooled among sampling dates. Data points from each sampling date are identified.



relative differences in otolith size and standard length among alevins at hatching were retained during the course of the experiment. Almost 70% of individuals that had small otoliths at hatching also had relatively small otoliths at the end of the experiment. No individuals that were classified as having small otoliths at hatching were reclassified as having relatively large otoliths at the end of the experiment. Similarly, 74% of alevins that were classified as having relatively large otoliths at hatching were also classified as having large otoliths after 20 days, while no individuals that had relatively large otoliths at hatching were reclassified as having small otoliths after 20 days. A similar result was found in the comparison of

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standard lengths of alevins at hatching and at the end of the experiment, although these results were less clear-cut. Between 44 and 52% of alevins that were classified as large, medium, or small on the basis of length remained in those categories at the end of the experiment.

Measurement error

To determine the contributions of measurement error to differing estimates of otolith size, data sets of remeasurements of the same individuals were analyzed as one-factor ANOVAs. The relative amount of variability contributed to the entire data set by differences in size among individuals and by replicate measurements of the same individual (measurement error) was then calculated using the method given in Snedecor and Cochran (1973, p. 280). In the analysis of otolith area, remeasurements of the same otoliths showed that measurement errors were small relative to differences among individuals and contributed only 2.44% of the total variability in the data set. In contrast, analysis of repeated measures of otolith radii showed that measurement error contributed 38% of the total variability in the data set. The greater levels of error associated with measurements of otolith radius, when compared with those of otolith area, were probably due to locating a consistent axis of measurement and aging errors. In these small individuals, the characteristic pattern of otolith growth found in older fish had not yet occurred, making it difficult to reliably measure the same axis within the otolith. In contrast, measurement of otolith area did not require age analysis and could be easily calculated.

Reanalysis of the videotapes showed that measurement error in estimates of standard length averaged 0.48 mm. These errors did not differ significantly among time periods and ranged from 0.56 to 0.31 mm (Table 2). As there was no major increase in the variability of standard lengths among alevins between hatching and 20 days, there was no consistent decline in the size of errors relative to the size range of alevins during the course of the experiment. Data sets for each time period were analyzed separately using one-factor ANOVAs and the relative contributions of measurement error at each time period calculated using the method given in Snedecor and Cochran (1973). These showed that the contribution of measurement error ranged between 10 and 65% of the total variability in the data sets.

The results of the simulation study (Table 3) demonstrated that levels of measurement error comparable with those that occurred in our study had a major influence on the strength of regression relationships calculated between otolith and fish size at emergence. Inclusion of levels of error in regression analyses associated with our measures of otolith radius and standard length resulted in a weak relationship (mean r^2 = 0.202) between otolith and fish size. Strong relationships between otolith and fish size ($r^2 > 0.75$) were not evident until levels of measurement error associated with otolith radius and standard length were reduced by 30 and 75%, respectively. When otolith area was used as a measure of otolith size the correlation between otolith and fish size substantially improved. At levels of error estimated to have occurred in our study, otolith area and fish length were moderately correlated (mean $r^2 = 0.475$). Reduction of measurement errors by 50% resulted in an increase in the mean value of r^2 to 0.786.

Table 1. Composition of three relative size categories (small, medium, and large) of otolith radius and standard length of individually identified salmon larvae at hatching and 20 days after hatching.

	Hatching		
20 days after hatching	Small	Medium	Large
Otolith radius			
Small	23	10	0
	69.7%	27.0%	
Medium	10	16	8
	30.3%	43.2%	25.8%
Large	0	11	23
		29.7%	74.2%
Standard length			
Small	14	16	6
	51.8%	31.8%	19.4%
Medium	7	17	9
	25.9%	43.6%	29.0%
Large	6	10	16
	22.2%	25.6%	51.6%

Note: For each size category at hatching, the table shows the number and percentage of fish that remained in the same category or were reclassified in other size categories 20 days later. Chi-square analyses for both size variables were significant (chi-square = 52.428, df = 4, $p = 0.001$ for otolith radius; chi-square = 11.36, df = 4, $p = 0.02$ for standard length).

Size selection

Comparison of frequency distributions of standard length at hatching of surviving alevins with those of fish that died during the 20-day experiment showed that mortality progressively removed the smallest members of the cohort, with alevins that were smallest at hatching being the first to die (Fig. 5). Size selection was not linked to growth rates. The mixed linear model comparison of growth trajectories revealed a significant interaction between alevin growth rate and time period of measurement ($F = 7.47$, df = 6, 186, $p < 0.0001$). Some dying fish were growing slower than the survivors of the 20-day experiment; others grew at similar rates. For example, those individuals that died between 5 and 10 days were not growing slower than surviving fish from 1 to 5 days after hatching. In contrast, fish that died 10–15 days after hatching were growing more slowly than surviving fish in the period from 0 to 10 days after hatching. Fish that died between 15 and 20 days after the start of the experiment displayed variable growth rates. These individuals grew more slowly than survivors up to 5 days after hatching, but then grew at the same rate as survivors until 15 days after hatching.

Discussion

Age

The otoliths of Atlantic salmon alevins provided a reliable record of age between hatching and emergence. Daily deposition of increments during this period has also been recorded in other salmonids including Arctic char (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) (Mosegaard and Titus 1987) and chinook salmon (*Oncorhynchus tshawytscha*) (Neilson and Geen 1982). However, Geffen (1983) suggested that in Atlantic salmon, entrainment to daily rates occurs only after emergence. In Geffen's study, alevins were raised under conditions

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Table 2. Errors associated with estimates of standard length measured from videotape records.

	SD (mm)		s^2	% variation
Hatching	0.537	Between	0.0918	
		Within	0.1703	65
5 days	0.477	Between	0.4849	
		Within	0.1174	24
10 days	0.563	Between	0.4300	
		Within	0.1680	39
15 days	0.505	Between	0.3074	
		Within	0.1493	48
20 days	0.316	Between	0.5068	
		Within	0.0538	10

Note: SD is the standard deviation of measurement error for each sampling date. These were derived from two independent measurements of standard length of each of 97 individuals at each sampling date. Data sets for each sampling date were analyzed using one-factor ANOVAs. Estimates of the variance components (s^2) associated with differences among individuals (between) and between replicate measures of the same individual (within) are shown. The percentage of variation in the data sets attributable to differences between replicate measurements of the same individual (measurement error) was calculated according to the formula given in Snedecor and Cochran (1973).

of constant temperature and regular cycles of light and dark, while in the present study, alevins were exposed to natural cycles of light and temperature. Such differences in experimental conditions might account for these apparently contradictory results, as Neilson and Geen (1982) have shown that daily cycles of temperature may be important for the entrainment of daily patterns of deposition in otoliths of young salmonids.

While it is possible that the daily pattern of increment deposition between hatching and emergence may have been influenced by the regular removal of alevins from the incubator for filming, this appears unlikely. The process of removal, filming, and replacement of alevins in the incubator was rapid and was usually completed in less than 10 min. No distinctive marks could be found on the otoliths that might have corresponded to these 5-day events.

Measurement error

Otolith and fish size of Atlantic salmon alevins were strongly correlated in the period between hatching and emergence. Modeling of the relationship between these variables suggested that variability in otolith size during this time could account for up to 98% of the variation in fish size. This strong relationship was not detected when linear regression was used to compare otolith and fish size for each of the 5-day sampling periods. Our simulation suggested that this was largely due to the measurement errors associated with size estimates. The use of otolith radius in the simulation resulted in a mean correlation coefficient between otolith and fish size of 0.2. About 21% of the r^2 values calculated by the simulation were lower than the actual coefficient calculated for alevins sampled 20 days after hatching (0.14). Substitution of otolith area as a measure of otolith size in the simulation resulted in a mean correlation of 0.475, a value similar to that calculated for data sets obtained from emergent alevins (0.44). These results imply that the changes in the strength of correlation coefficients that occurred when otolith area, rather than radius, was used as a size estimate in regression analyses were largely due to a reduction in measurement error.

Difficulties involved in aging of fish and measuring a consistent radius on the otolith may have contributed to the larger errors associated with measures of radii. In contrast, otolith area could be easily measured from whole otoliths and did not require age analysis. Secor and Dean (1992) and Neilson et al. (1985) observed that otolith area provides a more reliable estimate of size than otolith radius. The latter authors demonstrated that because of variation in the number and arrangement of primordia that fuse to form sagittae prior to hatching, area is a better estimate of otolith size than any unidimensional measure. They also suggested that variation in otolith size caused by variation in the number of fused primordia may weaken the otolith and fish size relationship at hatching, although no analysis was presented to support this proposition. West and Larkin (1987) found a change in the strength of the correlation coefficient from about 0.12 to 0.52 (which is comparable with the results of the present study) when radii measurements were replaced by estimates of sagittae area in regression analyses of fish and otolith size of juvenile sockeye salmon (*Oncorhynchus nerka*). We propose that the differing amounts of error associated with these measures of otolith size may account for this result.

The simulation demonstrated that reduction of errors in measurements of standard length had a greater effect on the strength of correlation coefficients than reductions in measurement errors of otolith size. Larger errors associated with standard lengths might account for the greater degree of reassortment of individuals among standard length categories than among otolith size categories in chi-square comparisons of fish at hatching and at the end of the experimental period. However, it is difficult to judge if the measures of fish and otolith size in our experiment were less reliable than those of previous studies, since few explicitly stated any estimate of error. As fish size was measured from videotapes, errors may have been greater than those associated with the direct measure of preserved individuals. Obviously, this latter technique is not possible in a longitudinal study of development.

Size selection

The source of mortality among individually contained alevins is unknown, although it was clearly size selective. Analysis of growth trajectories of fish that died during the experiment (reconstructed from videotape records) displayed no clear association between growth rate and mortality. Fish that died were smaller at hatching than those that survived, but in many cases were growing at the same rate as surviving fish during much of the experimental period. The removal of fish that were relatively small at hatching would have weakened the correlation between otolith and body size. Since individual otolith and fish size trajectories were parallel, size-selective mortality after hatching effectively truncated the length distributions of fish at emergence that could be used in regression analyses. This is also likely to have strongly influenced the importance of measurement error, by reducing the range of length differences among individuals. While the propagation by growth of size differences among survivors during the course of the experiment could have compensated for such an effect, this does not appear to be the case in our study. At

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hatching, the largest and smallest fish in the sample of 200 fry differed in standard length by 5.52 mm, while after 20 days, the 111 surviving individuals differed in standard length by a maximum of only 3.85 mm.

The effect of this sample truncation on the strength of regression relationships between fish and otolith size can be easily demonstrated. An expansion of the range of standard lengths of older fish can be achieved by randomly sampling 97 measurements (the total number of surviving individuals for which we had measurements of both standard length and otolith size) from the data sets collected 15 and 20 days after hatching. These subsamples were made without replacement (i.e., an individual selected at one time interval was not included in the subsample from the next time interval) to avoid any autocorrelation between measurements made on the same fish at different times. This resulted in a data set where the range in standard lengths was 4.4 mm. In the regression analysis of fish that survived until 20 days after hatching, the range in standard lengths was 3.85 mm and otolith and fish size were weakly correlated ($r^2 = 0.14$). When this subsampling was used to artificially expand the size range of fish used in the analysis (to 4.4 mm), the correlation between otolith and fish size increased to 0.66.

The effect of sample truncation on the ability of linear regression analyses to detect significant relationships is well recognized in growth and otolith studies of larval fish (Smale and Taylor 1987; Campana 1990). When body size and age are compared, sample truncation occurs when unbiased samples of all size groups in the population cannot be obtained as a result of gear selectivity (Murdoch and Doherty 1997) or due to size-selective mortality (Smale and Taylor 1987) or to growth effects on otolith size (Campana 1990). In such cases, sample truncation will result in the calculation of spurious regression lines where the slope and intercept are poor estimates of the actual values for the sample. Campana (1990) and Murdoch and Doherty (1997) recommended that the intercepts of lines should be constrained to biologically realistic values to overcome this problem. In our study, this effect of sample truncation can be illustrated by extrapolating the relationship between otolith and fish size to the average size of the alevins at hatching. For the data set collected at 20 days after hatching, the regression line gave a predicted length at hatching of 21.4 mm, a value that is obviously incorrect, since the average size of alevins at the beginning of our experiment was 15.7 mm. However, sample truncation, in this case due to size-selective mortality, will not only influence the slope and intercept of a regression line, but can also affect the strength of a correlation between the variables that are being compared. This will occur due to a reduction in the size range of individuals in the sample, which will increase the relative importance of measurement error.

In previous studies of Atlantic salmon, sample truncation has occurred as an artifact of the selection of experimental subjects. Metcalfe et al. (1992) compared otolith – fish size relationships in young salmon from two samples of individuals that were collected at different times. There was no significant correlation between otolith size and fork length at emergence in a small sample ($n = 19$) of fish collected immediately after emergence ($r^2 = 0.155$, fig. 1 in Metcalfe et al. 1992). A moderate correlation between these variables ($r^2 = 0.665$) was found in a larger sample of fish collected 3 months after emergence (fig. 2 in Metcalfe et al. 1992). Otolith radius ranged from 210 to 245 μm in the sample of fish collected at emergence, while in the sample collected at the later date, otolith radius at emergence ranged from 120 to 200 μm. Given that the ranges of otolith radii at emergence in these two samples do not overlap and that the range of radii in the fish collected at emergence was less than half that of fish collected 3 months after emergence, it is clear that the emergent fish used in their analysis were not representative of the population sampled at the later date. A second example of this problem is seen in Titus and Mosegaard (1991), where the young salmon used in their experiment were the progeny of a single pair of adults. While there is considerable variability in size of offspring among different parents, the progeny of one pair tend to be of a similar size (Titus and Mosegaard 1991). This artificial restriction of size ranges of emerging fish may have influenced the ability of regression analysis to detect significant relationships between otolith and fish size at emergence.

By taking a longitudinal approach to the comparison of otolith and fish size, we have shown that a strong relationship exists between these variables after hatching in young Atlantic salmon. This has important implications for studies that have proposed that selective mortality occurring in populations of

Table 3. Summary of results of a computer simulation of differing amounts of measurement error on the strength of correlations between otolith and fish size of salmon larvae at emergence.

	Length (mm)	r^2	SD	Min.
Radius (μm)				
2	0.12	0.828	0.024	0.727
	0.25	0.540	0.059	0.382
	0.50	0.232	0.069	0.046
5	0.12	0.762	0.033	0.669
	0.25	0.499	0.059	0.318
	0.50	0.221	0.065	0.043
7	0.12	0.699	0.039	0.586
	0.25	0.459	0.064	0.259
	0.50	0.202	0.065	0.017
Area (μm²)				
292	0.12	0.941	0.007	0.910
	0.25	0.789	0.027	0.703
	0.50	0.489	0.057	0.301
584	0.12	0.937	0.008	0.905
	0.25	0.786	0.027	0.683
	0.50	0.488	0.061	0.307
1170	0.12	0.921	0.011	0.891
	0.25	0.772	0.031	0.669
	0.50	0.475	0.060	0.278

Note: A detailed description of the simulation protocol is given in the Methods and materials section. A total of 500 data sets each containing 97 data pairs was used in each run of the simulation. Initially, the simulation used otolith radius as a measure of otolith size. The entire simulation was then repeated using total area as a measure of otolith size. The values shown for radius, area, and length are the standard deviations of the error component (mean = 0) that were added to the data sets calculated by the regression. The largest of each of these is equivalent to the error associated with size measurements in our experimental study. These were estimated from repeated measurements of the same individuals (see Methods and materials). The r^2 is the mean value calculated from the 500 data sets and SD is the standard deviation of this mean in each run of the simulation. The minimum r^2 calculated for the 500 data sets are shown.

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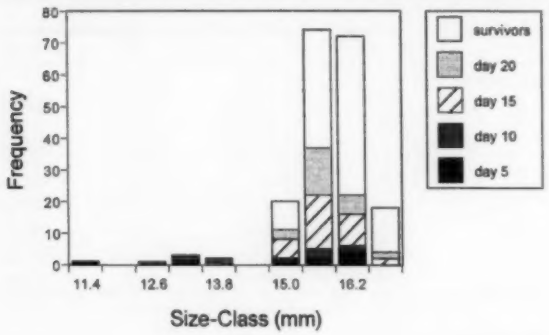
Fig. 5. Numbers of survivors and fish that were dead at days 5, 10, 15, and 20 as a function of size-class (standard length) at hatching.



young salmon in the period immediately following emergence operates on differences in metabolic rate that occur independently of variation in size among individuals (e.g., Mosegaard 1990; Titus and Mosegaard 1991; Metcalfe et al. 1992). These assume that the ability of regression analyses to detect correlations between otolith and fish size does not vary, irrespective of the ranges of size and age of fish examined in the study. Our work shows that this assumption is false. Small fish and their otoliths cannot be measured without error. The interaction of this error and sample truncation, due to size-selective mortality and (or) the selection of experimental subjects by investigators, may be able to explain the results of these previous studies, without the need to invoke processes of selection based on variables other than the size of young fish. However, traits such as size, growth, and metabolic rate are often highly correlated during the early life history of fish (Chambers et al. 1988; Hare and Cowen 1997). For this reason, our study does not exclude the possibility that selection for metabolic rate in young salmon does indeed occur, although determining the relative importance of any early life history trait during selection is likely to be difficult (Hare and Cowen 1997). However, changes in the apparent strength of the relationship between otolith and fish size in young salmon are not sufficient evidence to conclude metabolic rate selection without considering the importance of measurement error and sample truncation. If selection is size based (see Meeken et al. 1998), then the parallel nature of the otolith – fish size trajectories observed here indicates that it operates on differences in the relative sizes of alevins that are present at hatching.

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Does mortality select size or growth potential in young sockeye salmon (*Oncorhynchus nerka*)? A simulation based on otolith – fish size relationships[1]

M.G. Meekan, D.A.J. Ryan, J.J. Dodson, and S.P. Good

Abstract: There is conflicting evidence on the role of size-selective mortality in the demography of populations of young sockeye salmon (*Oncorhynchus nerka*). A previous field study suggested that increases in mean fork length and otolith size at emergence observed in a cohort of salmon between fry and smolt stages were due to size-selective mortality, such that fry with small fork lengths underwent higher mortality than larger individuals. However, a subsequent study used a simulation to show that such increases could not have been achieved by size-selective mortality without levels of survivorship of the cohort far lower than those that had been observed in the field. To account for field observations, the simulation study proposed that individuals with high metabolic rates had better survivorship, a process that was termed "selection for growth potential." Here, we use a simulation approach to show that size-selective mortality may result in shifts in mean fork length and otolith size at emergence comparable with those observed in the field at total mortalities within the range of estimates of natural values. The contrasting outcomes of earlier simulation work and the present study are probably due to the assumption by the former that otolith and fish size in young salmon were weakly correlated and the use of an inappropriate model of size-selective mortality. We conclude that size-selective mortality can explain the results of previous field studies and that little empirical evidence exists to support the hypothesis of selection for growth potential in cohorts of young salmon.

Résumé : Les données concernant le rôle de la mortalité sélective en fonction de la taille dans la démographie du jeune saumon rouge (*Oncorhynchus nerka*) sont contradictoires. Dans une étude sur le terrain effectuée antérieurement, on avait avancé une hypothèse selon laquelle l'accroissement de la longueur moyenne à la fourche et de la taille des otolithes à l'émergence observé dans une cohorte de saumons suivie depuis le stade de l'émergence des alevins jusqu'au stade du smolt était dû à la mortalité sélective en fonction de la taille, la mortalité étant plus lourde chez les alevins de faible longueur à la fourche que chez les sujets de plus grande taille. Or, dans une étude réalisée par la suite, on s'est servi d'un modèle de simulation pour montrer que l'accroissement des paramètres de longueur à la fourche et de taille d'otolithes ne pouvait pas s'expliquer par un phénomène de mortalité liée à la taille si les taux de survie de la cohorte n'étaient pas de beaucoup inférieurs à ceux mesurés sur le terrain. Dans cette étude en simulation, on expliquait les valeurs mesurées sur le terrain par une hypothèse selon laquelle le taux de survie est meilleur chez les saumons à métabolisme élevé en raison d'un phénomène de « sélection du potentiel de croissance ». Nous avons aussi eu recours à un modèle de simulation pour montrer que la mortalité liée à la taille peut causer des changements de longueur moyenne à la fourche et de taille des otolithes à l'émergence comparable à ceux observés sur le terrain lorsque la mortalité totale se trouve entre les valeurs estimatives limites de la mortalité naturelle. Si nos résultats diffèrent beaucoup de ceux obtenus dans l'étude antérieure, c'est probablement parce que dans cette dernière on a supposé une faible corrélation entre la taille des otolithes et la longueur à la fourche du jeune saumon, et l'on n'a pas utilisé le modèle approprié pour représenter la mortalité sélective par taille. Nous sommes parvenus à la conclusion que la mortalité sélective par taille peut expliquer les résultats des études antérieures faites sur le terrain, et qu'il n'y a guère de données empiriques pour étayer l'hypothèse d'une sélectivité favorisant le potentiel de croissance dans des cohortes de jeunes saumons.

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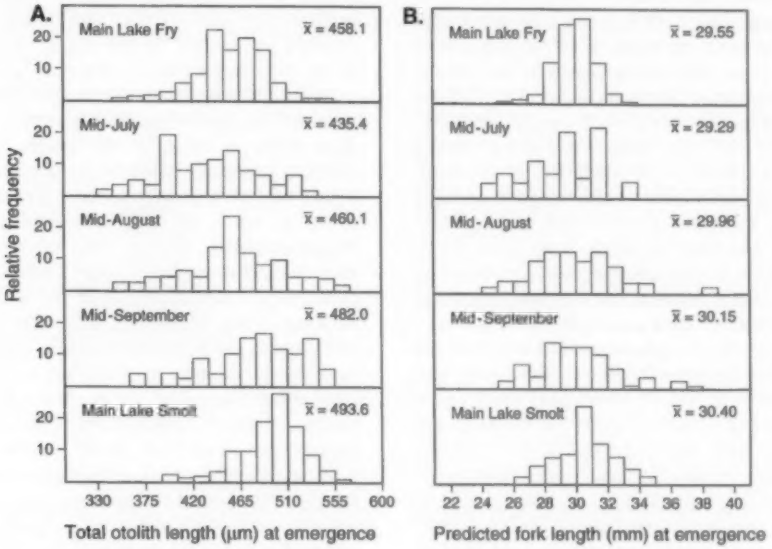
Fig. 1. Relative frequencies of (A) total otolith length and (B) predicted fork length at emergence for fry, juveniles in mid-July, mid-August, and mid-September, and 1+ smolts for the 1978 brood of sockeye salmon in Babine Lake, British Columbia. Data redrawn from figs. 6 and 7 in West and Larkin (1987). The regression equations used to generate predicted fork lengths are given in table 4 in West and Larkin (1987).



Introduction

Size- and (or) growth-selective mortality appears to have an important influence on the demography of fish populations (Bailey and Houde 1989). The analysis of age and growth information contained in otoliths can provide a convenient means of assessing the timing and extent of these processes, particularly when selection begins early in the life history of young fish (Campana 1996; Meekan and Fortier 1996).

West and Larkin (1987) used otolith analysis to examine the size-selective mortality of sockeye salmon (*Oncorhynchus nerka*) following emergence of fry from the redd in Babine Lake, British Columbia. They compared backcalculated distributions of otolith size at emergence from a cohort of fry that was sampled at emergence in the spring, at monthly intervals during the summer, and then again in the following spring, 1 year after emergence when the fry had become smolts. Comparison of these distributions showed a strong shift in mean otolith size at emergence with time (Fig. 1A, redrawn from West and Larkin 1987). Mean otolith length at emergence in the first spring sample was 458.1 μm. While there was little change in distributions of otolith size at the next sampling occasion in mid-July, there was a marked shift of distributions towards those individuals that had large otoliths at emergence by the end of the summer. This shift had become even more pronounced when the cohort was resampled as smolts, 1 year after emergence. At this time, the mean backcalculated otolith length at emergence of the cohort was 493.6 μm.

These measurements of otolith size were used by West and Larkin (1987) to reconstruct distributions of fork lengths at emergence (Fig. 1B). Backcalculations of fork length were made using linear (ln–ln) regressions between otolith size and fork length from fry collected at emergence and from fry collected on each sampling occasion. Comparison of distributions of fork length of the cohort displayed a shift towards larger sizes at emergence with time. In the initial spring sample, the mean fork length at emergence of the cohort was 29.55 mm, while 1 year later, the mean fork length at emergence of the cohort had increased to 30.40 mm.

West and Larkin (1987) attributed the change in mean otolith size and fork length at emergence in the cohort of young salmon to size-selective mortality. They argued that fry with smaller fork lengths at emergence underwent higher mortality than those with larger fork lengths, resulting in a shift of distributions of both fork and otolith lengths. Calculations of relative mortality rates provided some evidence to support this conclusion. Survival from the fry to smolt stages of salmon with otoliths below the mean length of 458.1 μm at emergence was 8.9%, while survival of salmon with otoliths above this mean value was estimated to be 63.6%. Although mortality estimated from the backcalculated fork lengths showed a similar pattern, survival of fish below and above the mean fork length of 29.55 mm was 27.2 and 43.3%, respectively. West and Larkin (1987) suggested that the difference between these estimates of relative mortality may have been due, at least in part, to errors introduced by the backcalculation procedure.

Mosegaard (1990) reanalysed the results of West and Larkin (1987) to examine the suggestion that size-selective mortality of fry could account for shifts in mean otolith length at emergence. Mosegaard simulated distributions of fork length at emergence from distributions of otolith length (Fig. 1A, top panel) using the regression between fork and otolith length at emergence shown in table 3 in West and Larkin (1987). Distributions of fork lengths at emergence were then subjected to differing degrees of selective mortality. Initially, a random sample of fry consisting of 10% of individuals below and 64% above the mean fork length of 29.74 mm was taken, which approximately corresponded to the survivorship

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pattern found in distributions of otolith length by West and Larkin (1987). This selection shifted mean fork length at emergence to 30.42 mm; however, mean otolith length only shifted to 467 μm. To determine what levels of mortality were required to obtain the much larger shift in mean otolith length (to 493.6 μm) that had been recorded by West and Larkin (1987), this simulation was then repeated with all individuals below a mean fork length undergoing mortality and all those above the mean surviving. Eight mean fork lengths were chosen, ranging from 30 to 33.5 mm. These simulations showed that to obtain a mean otolith length at emergence of 493.6 μm at the smolt stage, the cohort of young salmon had to undergo levels of total mortality that were up to an order of magnitude higher than those that had been recorded in the field. Thus, Mosegaard (1990) concluded that selective mortality based on fork length alone could not account for the shift in otolith length at emergence found by West and Larkin (1987).

As otolith growth may be strongly influenced by metabolic rate during the early life history of salmon (Mosegaard and Titus 1987; Mosegaard et al. 1988), Mosegaard (1990) proposed that shifts in otolith size may be largely due to a selection for metabolic rate. He argued that larger otolith size at emergence may reflect higher metabolic rates, rather than larger body size (fork length). High metabolic rate was thought to lead to higher growth rate, better competitive ability, higher survivorship, and ultimately larger body size at later life history stages. This process was later termed "selection for growth potential" by Titus and Mosegaard (1991).

To generate distributions of fork length, Mosegaard (1990) utilized a linear regression analysis that had been calculated between otolith and fork lengths at emergence by West and Larkin (1987). The relationship between these variables was weak (r^2 0.255). In a recent study, Meekan et al. (1998) demonstrated that when linear regression is used to compare otolith and body size in larval fish, the strength of the correlation between these variables is highly dependent on the choice of measures used to estimate size and on the age and size range of fish used in the analysis. This can be illustrated by comparing regression analyses calculated by West and Larkin (1987) for a sample of emergent fry. When area of the otolith was used as a measure of size rather than total length or radius of the otolith, there was up to a fivefold increase in strength of the correlation between otolith and fish size (see table 2 in West and Larkin 1987). Similarly, there was a large change in the strength of correlations that was dependent on the age of fry used in the regression analyses. At emergence, when the range of sizes in the cohort of fry was narrow, the correlation between somatic and otolith growth was poor (r^2 0.255). When the cohort was resampled at older ages and growth had propagated differences in size among individuals, correlations between otolith and fish size increased (r^2 0.31 to 0.64, table 4 in West and Larkin 1987).

These results have important implications for Mosegaard's simulation study. If otolith and fish size are strongly correlated, selection of individuals with large body sizes will result in an increase in mean body and otolith size at emergence in the cohort, since the surviving fish will tend to have large otoliths. When there is a poor correlation between these variables, selection will result in an increase in mean body size; however, the change in mean otolith size will be less marked, as the large, surviving fish may not necessarily have large otoliths. Under such conditions, a very high intensity of selection for larger body sizes will be required to produce shifts in mean otolith length of the cohort. Thus, the extreme levels of mortality that were required by Mosegaard's simulation to reproduce the results of West and Larkin (1987) may have been strongly influenced by the use of a regression relationship where otolith and fish size were weakly correlated.

A second potential problem with Mosegaard's simulations involved the means by which mortality was imposed. To produce shifts in mean otolith length at emergence, a step-function was imposed where all individuals smaller than mean values of fork length underwent a consistently high level of mortality, while all those individuals larger than mean fork length underwent a consistently low level of mortality. In contrast, natural mortality is more likely to operate as a smooth curve, with each size-class of fry within the cohort having differing levels of survivorship. Mortality, if size selective, will remove progressively fewer individuals as the size of fry increases. Evidence for variation in mortality rates among members of the cohort can be shown by comparing the distributions of otolith length in Fig. 1A. After 1 year, those individuals that had very small otoliths at emergence had disappeared, while some individuals were present in the final sample of the cohort that had larger otoliths at emergence than any that were found in the initial sample. One possible explanation for the sudden appearance of these fry with large otoliths is that at emergence, they were a very rare component of the cohort and unlikely to be sampled. Between fry and smolt stages, these fish underwent extremely low levels of mortality so that they became relatively more abundant and were thus included in samples at the smolt stage. This implies that survivorship of fry in the cohort varied from negligible to very high levels.

While Mosegaard (1990) conceded that a uniform level of survivorship among members of the cohort of differing fork lengths was unlikely to be realistic, he considered that his approach was conservative and that even more extreme levels of mortality would be required to produce shifts in mean otolith length if rates of survivorship differed among size-classes. However, this is not necessarily the case. A simulation where the same high level of mortality is applied to all individuals smaller than the mean size while all individuals larger than the mean undergo the same low level of mortality will result in the inclusion of small fry from the lower end of the distribution of fork lengths in calculations of means that may not have, under natural conditions, survived. Similarly, at the upper end of the distribution, large fry will undergo mortality where, in reality, survivorship may have been close to 100%. This source of error will not only apply to those fry that occur at the ends of the distribution of fork lengths and are a relatively rare component of the cohort, but also to those near mean values. At the center of the distribution, small changes in survivorship among size-classes of fork length will have a major effect on mean values, since the majority of individuals lie within this part of the distribution.

Here, we simulate size-selective mortality in a cohort of young sockeye salmon fry using field data published by West and Larkin (1987). The outcomes of these simulations are compared with those of Mosegaard (1990). We did this by firstly repeating the approach of Mosegaard (1990) where high and low levels of mortality were applied consistently to all

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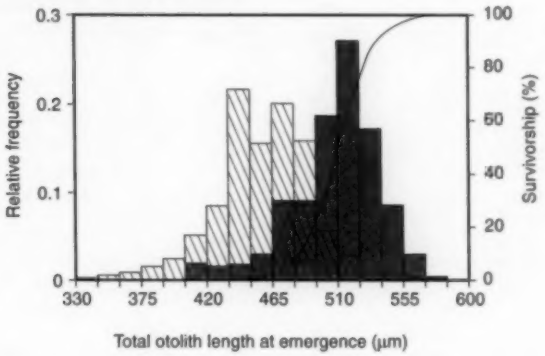
Fig. 2. Frequency distributions of total otolith length at emergence for fry (hatched bars) and 1+ smolts (shaded bars) from the 1978 brood of sockeye salmon in Babine Lake, British Columbia. Data redrawn from Fig. 1A (bottom panel, 1+ smolts) and table 1 in Mosegaard (1990). The survivorship curve is calculated by comparing frequency distributions using nonlinear regression (see Methods for details) is shown.



individuals below and above mean values of fork length in a cohort that had been generated using a regression between otolith and fork lengths calculated by West and Larkin (1987). The error used to generate distributions of fork lengths was altered in successive runs of the simulation so that the otolith – fish size relationship varied from weak (r^2 0.255) to strong (r^2 0.95).

Next, we estimated a survival curve from the distributions of otolith length at emergence that were obtained from successive samples by West and Larkin (1987) (Fig. 2). This survivorship curve was applied to distributions of fork lengths at emergence that had been generated using the regression between otolith and fork lengths calculated by West and Larkin (1987). The size of the error term in this regression was then reduced and the simulation repeated. These simulations allowed us to examine the effect of a survival curve (rather than a step-function where all individuals below mean fork lengths undergo the same high level of mortality, while those above the mean undergo the same low level of mortality) and variability in the strength of the otolith – fish size relationship on the amount of mortality required to produce shifts in mean fork and otolith lengths at emergence with time.

Methods

Generation of random observations of fork length from distributions of otolith length

We generated 1000 data sets of 400 otolith lengths from the frequency distribution of otolith lengths shown in Fig.1A (top panel) using the inverse transformation method (Naylor et al. 1966) (PROC RANUNI, SAS V6). The regression equation (table 3 in West and Larkin 1987)

$$\text{Fork length (fl)} = e^{1.52293 + 0.30493 \ln(x) + \text{error}}$$

where the error term was assumed to be a random normal variate with mean 0 and standard deviation (SD) 0.037 was then used to transform the simulated otolith lengths into fork lengths for all data sets. This resulted in 1000 data sets each with 400 simulated otolith and fork length measurements. The error of 0.037 was chosen to correspond to the SD used in simulations by Mosegaard (1990). This error was then progressively reduced to 0.02, 0.015, 0.005, and 0 and a further 1000 data sets generated for each level of error. These SDs corresponded to (ln–ln) regression equations between otolith and fork length where the average r^2 = 0.54, 0.68, 0.95, and 1, respectively.

Simulation of size-selective mortality using a step-function

Comparisons of distributions of otolith and fork lengths of fry collected at emergence and at smolt stages were used by West and Larkin (1987) to calculate relative survivorships of fry above and below mean values of fork length. These estimates were then used by Mosegaard (1990) to simulate size-selective mortality in a cohort of fry. In his study, constant proportions of individuals above and below mean values were removed from distributions of fork lengths. We repeated his simulation protocol by randomly removing fish from the data sets in the following manner. Each of the simulated pairs of data was randomly assigned a number between 0 and 1 using the RANUNI procedure in SAS V6. In the first run of the simulation, fish that had fork lengths smaller than the mean had a survivorship of 27%, while those above the mean had a survivorship of 43%. Consequently, all data pairs that had been assigned a number larger than 0.27 and had fork lengths smaller than the mean value were removed, resulting in 27% survivorship for this part of the cohort. All data pairs that had been assigned a value larger than 0.43 and had fork lengths greater than the mean were also removed, resulting in 43% survivorship for this upper part of the cohort. Mean otolith and fork lengths of the survivors were then obtained for each data set. These values were averaged among the 1000 data sets and SDs of the means were calculated. The simulation was then repeated for the fork length data sets that had been generated with differing levels of error.

The entire process was then repeated using survivorships of 10 and 64% below and above mean fork lengths, respectively. These levels of survivorship corresponded to the estimates calculated by West and Larkin (1987) and used in simulations of size-selective mortality by Mosegaard (1990).

Estimation of the survival curve

It was assumed that survival due to size-selective mortality could be approximated by a monotonic increasing, S-shaped curve described by

$$s(x) = e^a/(1 + e^a)$$

where $s(x)$ is the probability that a fry with an otolith length of x will survive to the smolt stage and $a = \beta_0 + \beta_1 x$ where β_0 and β_1 represent the location and slope of the curve, respectively. Using PROC NLIN (SAS V6), the following relationship was used to estimate $s(x)$:

$$g_i(x) = (s(x)f_i(x))/c_i$$

where c_i is the sum of $s(x)f_i(x)$ over all otolith size-classes in the distribution, $g_i(x)$ is the observed percentage of fry surviving to the smolt stage for size-class i (Fig. 2), and $f_i(x)$ is the observed percentage of fry at emergence for size-class i (Fig. 2). The parameter estimates for β_0 and β_1 calculated by the nonlinear regression were –41.907 58 and 0.081 90, respectively, and the midpoint of the curve lay at 511.69 μm.

Application of the survivorship curve to distributions of fork lengths

The nonlinear regression resulted in the parameter estimates for the curve being expressed in terms of otolith radius. To apply the curve to distributions of fork length, these values were transformed to fork lengths using the regression equation (table 3 in West and Larkin 1987) without an error term, resulting in the following survivorship curve:

$$s(y) = e^a/(1 + e^a)$$

where $s(y)$ is the probability that a fry with a fork length of y will survive to the smolt stage and $a = -41.90758 + 0.00056y^{3.28}$. In

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Table 1. Mean otolith and fork lengths at emergence of a cohort of sockeye salmon after constant survivorships were applied to distributions of fork length below and above mean values.

	27% below, 43% above mean				10% below, 64% above mean			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Error = 0.005, $r^2 = 0.95$								
Otolith length (μm)	464.31	2.64	455.74	472.31	476.58	2.14	470.15	484.61
Fork length (mm)	29.81	0.05	29.62	29.98	30.07	0.04	29.95	30.24
Survivorship (%)	34.84	2.32	27.00	42.50	36.81	2.46	28.00	43.75
Error = 0.015, $r^2 = 0.68$								
Otolith length (μm)	463.32	2.75	454.63	471.27	473.58	2.26	466.45	479.81
Fork length (mm)	29.84	0.06	29.63	30.04	30.15	0.05	30.01	30.32
Survivorship (%)	34.83	2.42	27.50	42.50	36.55	2.41	29.5	44.25
Error = 0.02, $r^2 = 0.54$								
Otolith length (μm)	462.82	2.58	455.33	471.62	471.92	2.30	465.41	479.07
Fork length (mm)	29.86	0.07	29.63	30.09	30.2	0.05	30.03	30.37
Survivorship (%)	34.84	2.30	27.75	42.25	36.47	2.43	29.50	43.50
Error = 0.037, $r^2 = 0.26$								
Otolith length (μm)	461.48	2.71	451.36	470.14	467.94	2.48	460.24	476.03
Fork length (mm)	29.95	0.10	29.61	30.28	30.47	0.08	30.19	30.77
Survivorship (%)	34.84	2.45	28.00	43.75	36.36	2.44	29.50	44.00

Note: Survivorships used in the simulations are shown in the column headings. Distributions of fork lengths were generated using a linear (ln–ln) regression between otolith and fork lengths calculated by West and Larkin (1987). The error levels used in successive simulations are shown. Means and SDs were derived from 1000 distributions of 400 individuals.

addition to $s(y)$, a random uniform variate (l_i) was generated using RANUNI (SAS V6) and individual fish were categorized as survivors if l_i was less than $s(y)$; otherwise the fish was removed from the data set. Mean otolith and fork lengths of the survivors were obtained and these values were averaged among the 1000 data sets and SDs of the means calculated. The simulation was then repeated for the fork length data sets that had been generated with differing levels of error.

The nonlinear regression outlined above resulted in fish with fork lengths of 30.72 mm lying at the midpoint of the survivorship curve. These individuals had a 50% chance of being removed from the data sets during the simulation. Fish with fork lengths larger than this value had a greater chance of survivorship, while fish with lengths smaller than this value had a lower chance of survivorship. However, since this survivorship curve was calculated from single samples of the population, it represented only one possible estimate of the actual pattern of survivorship of the cohort from fry to smolt stages. Consequently, we altered slopes and intercepts to generate three new curves, each of which lay further to the left of the original survivorship curve on the x-axis (see Fig. 2) with midpoints at 30.5, 29.5, and 28.5 mm, respectively. These new curves were then applied to the data sets of fork length that had been generated with the differing errors shown above. This allowed us to examine the effect of differences in the position of the survivorship curve on shifts in otolith size at emergence between fry and smolt stages.

Results

Simulated data sets of otolith and fork lengths at emergence had mean values of 458.65 μm (± 1.55 SD) and 29.70 mm (± 0.04 SD), respectively. Changes in mean otolith and fork lengths at emergence in simulations where constant proportions of individuals below and above mean values of fork length underwent mortality are shown in Table 1. These survivorships (27 and 43%, 10 and 64% of fry below and above mean fork lengths) corresponded to those calculated by West and Larkin (1987) and used in simulations by Mosegaard (1990). When they were applied to distributions of fork length generated with the same large error (0.037) used by Mosegaard (1990), the results were identical to those calculated by his simulation (Table 1, bottom block of data). For example, at survivorships of 27 and 43% below and above mean values of fork length, Mosegaard (1990) calculated that mean otolith length shifted to 461 μm, while mean fork length shifted to 29.97 mm. Using the same levels of error (0.037) and survivorship, our simulation produced shifts in mean otolith and fork length to 461.48 μm and 29.95 mm, respectively. At levels of survivorship of 10 and 64% above and below mean values, Mosegaard's simulation resulted in shifts of mean otolith length to 467 μm and a shift in fork length to 30.42 mm, while our simulation produced shifts in mean otolith and fork lengths to 467.94 μm and 30.47 mm, respectively.

When our simulation applied mortality as a step-function to data sets of fork length that had been generated using smaller errors, so that fish and otolith size were more closely correlated, the resulting shifts in mean otolith and fish size were still smaller than those found by West and Larkin (1987). The application of uniform survivorships of 10 and 64% below and above mean fork length to data sets where otolith and fish size were tightly coupled (error = 0.005, equivalent to $r^2 = 0.95$) shifted mean otolith length to only 476.6 μm (± 2.14 SD, Table 1), a value well below the shift to 493 μm that was recorded by West and Larkin (1987).

Shifts in mean otolith and fork length after the survivorship curve (Fig. 2) was applied to the distributions of fork lengths at emergence are shown in Table 2. When the curve was imposed on the fork length data set that had been generated without error, mean otolith size shifted to 504.81 μm (± 4.48 SD). When applied to distributions of fork length that were generated using a relatively large error (0.037), there was a shift in mean fork length to 31.34 mm (± 0.07 SD) and a

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Table 2. Mean otolith and fork lengths at emergence of a cohort of sockeye salmon after application of a survivorship curve to distributions of fork lengths that were generated using a linear (ln–ln) regression between otolith and fork lengths calculated by West and Larkin (1987).

	Mean	SD	Min.	Max.
Error = 0, $r^2 = 1$				
Otolith length (μm)	504.81	4.48	491.47	523.05
Fork length (mm)	30.58	0.08	30.32	30.94
Survivorship (%)	7.76	1.27	3.75	11.5
Error = 0.005, $r^2 = 0.95$				
Otolith length (μm)	503.21	4.24	490.80	519.44
Fork length (mm)	30.61	0.08	30.38	30.93
Survivorship (%)	8.24	1.31	4.50	12.25
Error = 0.015, $r^2 = 0.68$				
Otolith length (μm)	494.22	3.58	483.14	509.92
Fork length (mm)	30.80	0.07	30.59	31.02
Survivorship (%)	11.72	1.62	7.25	17.25
Error = 0.02, $r^2 = 0.54$				
Otolith length (μm)	489.37	3.35	478.62	501.52
Fork length (mm)	30.92	0.07	30.71	31.14
Survivorship (%)	14.24	1.80	9.25	20.50
Error = 0.037, $r^2 = 0.26$				
Otolith length (μm)	477.59	2.87	467.14	487.18
Fork length (mm)	31.34	0.07	31.07	31.59
Survivorship (%)	22.57	2.20	15.7	31.75

Note: Distributions were generated using errors of 0, 0.005, 0.015, 0.02, and 0.37. Means and SDs were derived from 1000 distributions of 400 individuals. The equation for the survivorship curve is given in the Methods section.

corresponding shift in otolith length to 477.59 μm (± 2.87 SD). Application of the survival curve to distributions of fork length that had been generated using a small error (0.005) resulted in a smaller shift in mean fork length to 30.61 mm (± 0.08 SD) but a much greater shift in mean otolith length to 503.21 μm (± 4.24 SD).

The imposition of the survivorship curve on distributions of fork lengths that had been generated without an error component gave a total survivorship of the cohort from fry to smolt stages of 7.8%. When the survivorship curve was applied to the distributions of fork lengths that had been generated with error components, survivorship increased (Table 2). Distributions that were generated using a medium amount of error (0.015), corresponding to a relationship between otolith and fork length where $r^2 = 0.68$, showed a shift in mean otolith length equivalent to that found by West and Larkin (1987). This shift was achieved with a survivorship of 11.72% (± 1.62 SD). Where a greater level of error (0.02, corresponding to an r^2 of 0.54) was introduced into the generation of fork length distributions, application of the survivorship curve resulted in a shift in mean otolith length that was slightly smaller than the value recorded by West and Larkin (1987), but still within 1.5 SD of their estimate. This shift was obtained with a total survivorship of 14.24% (± 1.80 SD).

By altering the slope and intercept of the survivorship curve derived from the comparison of otolith size distributions, three new curves were produced that allowed progressively greater survivorship of the cohort between fry and smolt stages. Application of these new curves to distributions of fork length generated using the differing levels of error usually resulted in smaller shifts in mean otolith size at emergence than the shift found when the original curve was used in the simulation (Table 3). When the first of these curves was applied to data sets of fork length generated with a level of error of 0.005, the resultant shifts in mean otolith and fork lengths were indistinguishable from those recorded by West and Larkin (1987). These were achieved with a survivorship of 15.11% (± 1.87 SD). When this curve was applied to fork lengths generated with an error of 0.02, fork length shifted to 30.74 mm (± 0.05 SD) and otolith length to 484.90 μm (± 2.77 SD). In this case, about 20% of the cohort survived to the smolt stage. Application of the remaining survivorship curves resulted in shifts of mean otolith length to values that were considerably smaller than that recorded by West and Larkin (1987), irrespective of the amount of error used in the generation of distributions of fork length.

Discussion

Varying the strength of the regression relationship between otolith and fish size and the imposition of a survivorship curve on distributions of fork length had major effects on the amount of size-selective mortality required to produce shifts in mean otolith length at emergence in a cohort of young sockeye salmon. We were able to reproduce the results of Mosegaard's (1990) study by repeating his simulation protocol, where mortality was applied as a step-function to distributions of fork length that had been generated using a large amount of error (0.037). When the strength of the correlation between otolith and fish size was increased and mortality again applied as a step-function to fork lengths, our simulation achieved shifts in mean otolith length at emergence that were greater than those found in Mosegaard's (1990) study, but were still considerably smaller than the shift recorded by West and Larkin (1987). However, the use of a survivorship curve, rather than removal of constant fractions of the cohort above and below mean values of fork length, resulted in shifts in mean otolith length that were often greater than the value recorded in the Babine Lake system.

The survivorship curve enabled us to create shifts in mean otolith length comparable with those described by West and Larkin (1987) at survivorships ranging from 7.6 to 20% of the cohort between fry and smolt stages. Prior to the study of West and Larkin (1987), McDonald and Hume (1984) monitored survival of sockeye salmon in Babine Lake over a 16-year period. Based on estimates of numbers of fry entering and smolts exiting the lake, they calculated that survival rates between these stages ranged from 7.5 to 49.9% (mean 30.44, SD 10.82). Thus, our shifts in mean otolith length at emergence were achieved at survivorships within the range of values that have been observed under natural conditions. In contrast, simulations of size-selective mortality by Mosegaard (1990) were unable to replicate the change in mean otolith length found by West and Larkin (1987) without the imposition of mortalities that were up to an order of magnitude greater than those recorded by McDonald and Hume (1984). Our simulations show that this result was probably due to the assumption by Mosegaard (1990) that mortality would remove constant

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Table 3. Mean otolith and fork lengths at emergence of a cohort of sockeye salmon after application of three survivorship curves to distributions of fork lengths that were generated using a linear (ln–ln) regression between otolith and fork lengths calculated by West and Larkin (1987).

	Curve 1		Curve 2		Curve 3	
	Mean	SD	Mean	SD	Mean	SD
Error = 0, $r^2 = 1$						
Otolith length (μm)	495.71	3.06	472.91	1.71	463.81	1.52
Fork length (mm)	30.41	0.06	29.98	0.03	29.80	0.03
Survivorship (%)	14.42	1.76	58.01	2.42	84.90	1.78
Error = 0.005, $r^2 = 0.95$						
Otolith length (μm)	494.66	3.13	472.80	1.71	463.80	1.52
Fork length (mm)	30.44	0.05	29.99	0.03	29.80	0.03
Survivorship (%)	15.11	1.87	57.89	2.45	84.74	1.79
Error = 0.015, $r^2 = 0.68$						
Otolith length (μm)	488.50	2.90	471.80	1.72	463.85	1.53
Fork length (mm)	30.62	0.06	30.09	0.04	29.85	0.03
Survivorship (%)	18.44	1.96	57.05	2.45	83.35	1.88
Error = 0.02, $r^2 = 0.54$						
Otolith length (μm)	484.90	2.77	471.14	1.78	463.86	1.60
Fork length (mm)	30.74	0.05	30.17	0.04	29.88	0.04
Survivorship (%)	20.72	1.95	56.50	2.49	82.26	1.90
Error = 0.037, $r^2 = 0.26$						
Otolith length (μm)	475.87	2.61	468.67	1.94	463.73	1.65
Fork length (mm)	31.17	0.07	30.52	0.06	30.10	0.06
Survivorship (%)	27.99	2.28	54.89	2.51	77.46	2.06

Note: Distributions were generated using errors of 0, 0.005, 0.015, 0.02, and 0.37. Means and SDs were derived from 1000 distributions of 400 individuals. Each curve lay to the left on the *x*-axis of the original survivorship curve shown in Fig. 2. Midpoints for curves 1–3 were 30.5, 29.5, and 28.5 mm, respectively.

proportions of the cohort above and below mean values of fork length and that otolith and fish size were weakly correlated (r^2 0.255).

When survivorship curves were imposed on distributions of fork lengths, it was not necessary to assume that fish and otolith size were strongly correlated ($r^2 > 0.80$) to achieve a shift in mean otolith length that was comparable with that recorded in the field by West and Larkin (1987). Application of the size-selective survivorship curve to fork length distributions that were generated using an error of 0.02, equivalent to a regression relationship where otolith and fish size were moderately correlated (r^2 0.54), resulted in a shift in mean otolith length at emergence to 489 μm and a survivorship of 14% of the cohort from fry to smolt stages. Regression analyses calculated by West and Larkin (1987) between otolith size and fork length of fry at emergence show that a correlation of this strength could be obtained by using area of the otolith as a measure of size, rather than total length. Such changes in the strength of regression relationships probably reflect the relative amounts of measurement error that are associated with differing estimates of otolith size (Meekan et al. 1998).

Our results show that it is not necessary to invoke mortality based on selection for any trait of individuals other than body size (fork length) to account for the results of West and Larkin (1987). Differences in size among individuals are known to have an important influence on dominance hierarchies and life history patterns at older ages in salmonids (Fausch 1984; Metcalfe et al. 1992). West and Larkin (1987) suggested that size differences among members of a cohort of young salmon are established at the time of emergence and are then propagated by growth. Meekan et al. (1998) have shown that such variation may in turn be generated from differences among individuals that are present at hatching. This implies that the traits upon which selective mortality will act may be established at hatching.

There is little direct evidence of selective mortality of salmon fry as a function of variability in metabolic rates among individuals rather than body size (selection for growth potential). With the exception of the simulation study of Mosegaard (1990), the only other published evidence that this process might occur has been the work of Titus and Mosegaard (1991). In their study, brown trout (*Salmo trutta*) fry that were raised in tanks underwent selective mortality so that the surviving individuals were those that had large otoliths at emergence. From a simulation based on growth and starvation rates, they concluded that this mortality was unlikely to be size selective. As in Mosegaard's (1990) study, a weak relationship (r^2 0.06) between otolith and fish size was a central assumption of their simulation. However, the strength of correlations between these variables recorded by Titus and Mosegaard (1991) may have been influenced by the use of dry weights as a measure of fish size. At emergence, it is likely that traits of fry other than body size such as gut contents, condition, and the amount of yolk sac absorption contribute to measures of dry weight. Furthermore, the weight of remaining yolk at emergence should be negatively correlated with fish size. Due to such

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confounding influences, weak correlations between otolith and fish size might be expected when dry weight is used as a body size measure at emergence. The present study demonstrates that this will have an important influence on the amount of size-selective mortality that is required to produce shifts in mean otolith size in a cohort of young fish. Despite these caveats, it is still possible that metabolic rates may contribute to the process of selective mortality that occurs during the early life history of salmonid fishes, since growth and metabolic rates will be strongly correlated.

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Distribution of hoki (*Macruronus novaezelandiae*) eggs, larvae, and adults off Westland, New Zealand, and the design of an egg production survey to estimate hoki biomass

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Abstract: The winter spawning aggregations of hoki (*Macruronus novaezelandiae*) off the Westland coast, South Island, support New Zealand s largest trawl fishery. The distribution and abundance of hoki eggs, larvae, and adults from ichthyoplankton and acoustic surveys made during the period 1978–1993 were used to describe the location and timing of hoki spawning off Westland. Hoki spawned primarily in south Westland, around Hokitika Canyon, and south along the outer shelf and slope to Cook Canyon, although spawning biomass appeared to increase in north Westland later in the season. Older stage hoki larvae occurred shoreward of eggs and newly hatched larvae, suggesting onshore advection. The data were used to designate spatial and temporal egg sampling strata that significantly increased egg abundance estimate precision. The egg data were used to estimate annual egg production and, combined with data on fecundity and recruited proportion spawning, to estimate virgin biomass. The historical biomass estimate (1.48 million tonnes, CV = 29.4%) was probably biased down because the plankton tows were too shallow. It appears that a future egg production survey, using appropriate plankton sampling depths, could provide a biomass estimate of Westland spawning hoki sufficiently accurate and precise for management purposes.

Résumé : Les groupements de hokis (*Macruronus novaezelandiae*) qui frayent en hiver, au large de la côte du Westland de l île du Sud, alimentent la plus importante pêche au chalut de la Nouvelle-Zélande. Les données sur la distribution et l abondance des oeufs, des larves et des adultes de hoki, provenant de relevés de l ichtyoplancton et de relevés acoustiques effectués entre 1978 et 1993, ont été utilisées pour décrire l emplacement et la période de fraye de cette espèce au large du Westland. Les hokis frayent principalement au sud du Westland, à proximité du canyon Hokitika, et au sud, le long de la partie extérieure de la plate-forme et du talus continental jusqu au canyon Cook; mais il y aurait eu accroissement de la biomasse des reproducteurs dans le nord du Westland plus tard dans la saison. Les larves de hoki à un stade plus avancé se trouvaient plus près de la côte que les oeufs et les larves nouvellement écloses, ce qui serait le signe d une advection côtière. Les données ont servi à établir des strates d échantillonnage spatio-temporelles des oeufs, ce qui a augmenté considérablement la précision de l estimation de l abondance des oeufs. Les données sur les oeufs ont servi à évaluer la production annuelle d oeufs et, combinées aux données sur la fécondité et sur la proportion de géniteurs qui sont recrutés, à évaluer la biomasse vierge. L évaluation de la biomasse des années antérieures (1,48 million tonnes, CV = 29,4%) était probablement biaisée à la baisse parce que les traits de filets à plancton ont été effectués trop près de la surface. Un relevé futur de la production d oeufs, utilisant des profondeurs appropriées d échantillonnage du plancton, pourrait fournir une estimation de la biomasse de la population de géniteurs du Westland suffisamment juste et précise aux fins de la gestion.

[Traduit par la Rédaction]

Introduction

The winter spawning aggregations of hoki (*Macruronus novaezelandiae*) off the Westland, South Island, coast (Fig. 1) support New Zealand s largest trawl fishery, with landings of 76 000 tonnes in 1995–1996 (Annala and Sullivan 1997). These fish comprise the spawners of the "western stock" of hoki (Annala and Sullivan 1997) and they migrate to Westland in late June, spawn through July and August, and disperse in September (Patchell 1982; Sullivan 1991; Langley 1993; Zeldis 1993). The spatial and temporal distributions of hoki spawning biomass within Westland have not been well described. However, the distribution and movement of the hoki fishing fleet along the Westland coast during the spawning season indicate that aggregations of spawning hoki are centred primarily around the Hokitika Canyon region in late June and July (Hurst 1987). By mid-August, much of the fleet moves north to fish over the outer shelf, northwest of Westport (Hurst 1987), before the fish disperse at the end of the spawning season.

Hoki migrate into and out of the west coast spawning grounds during the spawning season, but the rate and extent of

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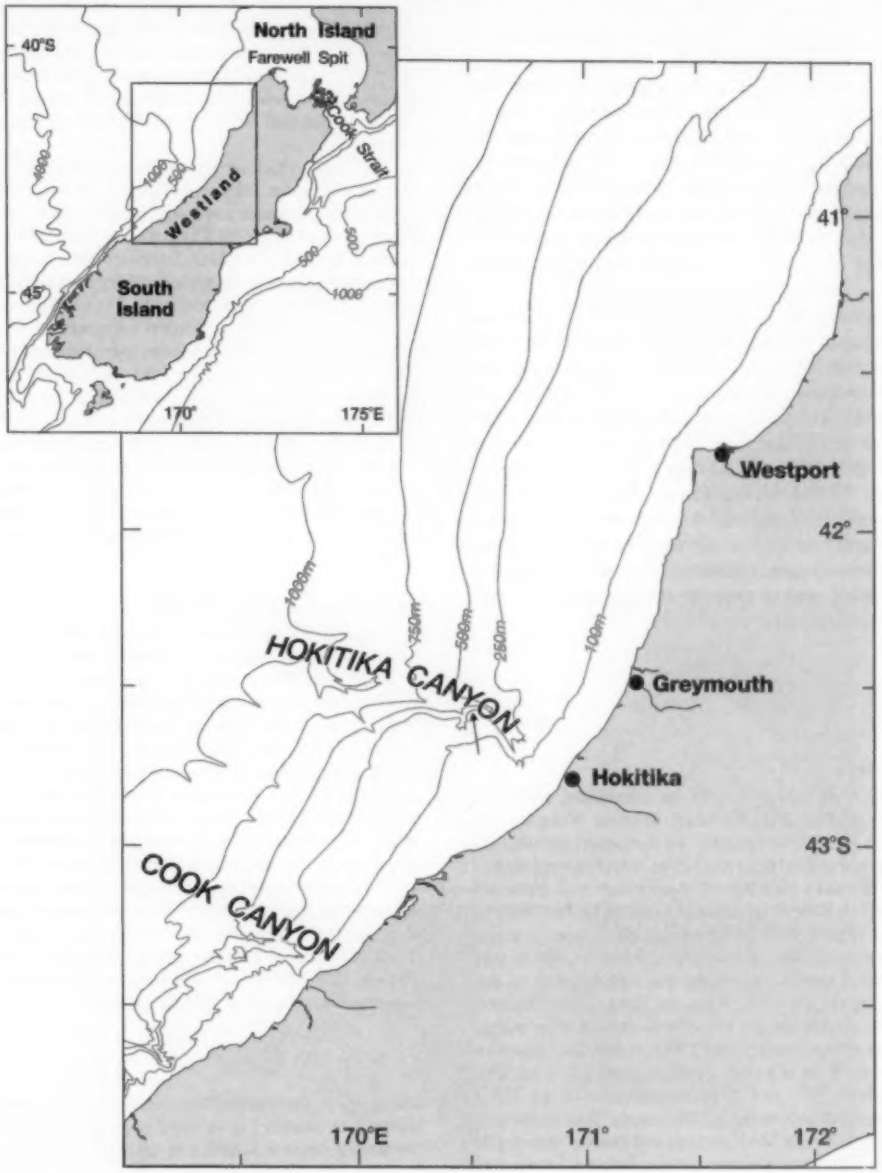
Fig. 1. Place names and bathymetry in Westland, South Island, New Zealand.



this "turnover" are not well known. A gradual reduction in the mean length of hoki as the spawning season progresses (Sullivan 1991) suggests that the spawning population is in a continual state of flux, and the low numbers of spent fish caught during the spawning season suggest that fish leave the spawning grounds soon after they complete spawning (Langley 1993). The minimum time that female hoki spend on the spawning grounds has been estimated as 20–27 days, as this is the length of time between the first occurrences of immediately prespawning and spent fish in trawl catches (Langley 1993). As this minimum time is much shorter than the population spawning season of about 50 days, clearly, turnover occurs.

From a stock assessment standpoint, turnover will cause an underestimate of absolute spawning stock biomass if "snapshot" methods of biomass estimation are used (e.g., trawl, acoustic, or daily egg production surveys). For this reason, the absolute biomass of hoki in the western stock is currently estimated using time series of relative abundance indices (trawl and acoustic surveys, catch and effort data, and relative recruit strengths) within a stock reduction analysis (Sullivan et al. 1995). However, confidence in this biomass estimate is not high (Sullivan et al. 1995) and would be significantly improved if a survey estimate of absolute biomass of Westland spawning hoki became available (Cordue 1996).

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The annual egg production method (AEPM) (Saville 1964; Hunter and Lo 1993; Picquelle and Megrey 1993; Zeldis 1993) can provide a survey estimate of absolute biomass because it circumvents the turnover problem. It estimates spawning biomass from a series of surveys that together estimate the integrated egg production over the entire spawning season. However, the AEPM is very demanding on ship and analysis time when conducted at intensities that yield estimates of biomass precise and accurate enough to be useful for stock management. Therefore, it is important to optimise the design and forecast the size, number, and timing of egg production surveys that would be required to achieve this management goal.

The first objective of the present study was to describe the temporal and spatial distributions of hoki spawning biomass off Westland, based on the winter distribution of hoki eggs and larvae from ichthyoplankton surveys in the winters of 1978–1981 and 1987–1988 and on distributions of hoki adult biomass from acoustic surveys from 1989 to 1993. The second objective was to use these historical data to provide an indicative design for an AEPM survey of the absolute spawning biomass of hoki off Westland and to evaluate the potential precision and accuracy of such a survey. The final objective was to use the historical data to estimate a virgin biomass for the western hoki stock and to evaluate the potential bias and precision of such an estimate.

Methods

Ichthyoplankton surveys

Grids of stations were surveyed over the continental shelf and slope during winter months (May–October) between Westport and Cook Canyon (Figs. 1 and 2) to describe the horizontal distribution and seasonal occurrence of hoki eggs and larvae. Surveys were undertaken by the New Zealand Ministry of Agriculture and Fisheries (MAF) in 1978–1981 and the New Zealand Oceanographic Institute (NZOI) in 1987 and 1988. For the MAF surveys the station coverage in each survey depended on time and weather constraints, and so was variable. For the NZOI surveys, sampling was concentrated on the southern part of the survey area only. Eggs and larvae were collected at each station with a double oblique tow of a 61-cm-diameter bongo zooplankton sampler (Posgay and Marak 1980) to near the bottom or a maximum depth of 200 m at a ship speed of about 2.5–3 kn. The frames were fitted with 500- and 300-μm-mesh nets in the MAF surveys and 320-μm-mesh nets in the NZOI surveys. Tow depth was estimated by wire angle for the MAF surveys and from a time–depth recorder for the NZOI surveys. Volume filtered during all tows was determined using a General Oceanics flowmeter centred in the mouth of each net. All samples were preserved with 5% formalin in seawater.

In the laboratory, all hoki eggs and larvae were identified and counted. When the numbers of eggs and larvae in the samples were large, counts were made from subsamples separated with a Folsom plankton splitter. Larval length (tip of the snout to the end of the notochord or hypural elements) was determined for larvae collected in only the NZOI surveys by measurement under a dissecting microscope fitted with an ocular micrometer. All larvae were measured, or a random subsample of 50 was measured if the number of larvae in the sample exceeded 50. The length of damaged larvae was estimated from the linear relationship between the tail length from the centre of the ventral melanophore (TM) and total length (TL) for undamaged larvae, whereby TL (millimetres) = 1.731TM (millimetres) + 0.893 (r = 0.88, n = 54).

Acoustic surveys

Acoustic surveys of spawning hoki off the Westland coast were made each year from 1984 to 1993, except in 1986. The evolution of the systems and designs from 1985 to 1991 is described in Coombs and Cordue (1995). During the period from 1989 to 1993 (for which results are considered here) the survey design changed little, and within-season and between-year comparisons of the results can be made.

The 1989–1993 surveys followed the stratified random, parallel transect design of Jolly and Hampton (1990). The survey area was divided into six strata covering bottom depths from 300 to 650 m (in strata north of Hokitika Canyon) and from 250 to 750 m (in strata south of Hokitika Canyon). Transects were orientated perpendicular to the depth contours, as hoki tend to aggregate along a depth contour. Details of the methods used to calculate average hoki density for each transect and overall hoki biomass estimates are given in Coombs and Cordue (1995). Transect densities were obtained using standard echo integration (Burczynski 1979; Johannesson and Mitson 1983) with an estimate of mean hoki target strength obtained from a cylindrical swim bladder model (Coombs and Cordue 1995). Biomass estimates for each stratum were obtained by multiplying the average transect density by the stratum area (after each transect density was weighted by the length of the transect). The estimate of hoki target strength is not considered reliable, but the estimates of biomass can be used in a relative sense to enable comparisons within seasons and between years.

Egg production survey design

The historical egg survey data were used to design an optimised egg production survey in the following way.

(*i*) The number of eggs per square metre of sea surface area was estimated for each station by multiplying the number of eggs per cubic metre at the station by the maximum depth of sampling at the station (about 200 m) or the bottom depth, if the bottom was <200 m deep. These densities were plotted on a single map of the South Island west coast and stratum boundaries were identified by finding (by eye) station groupings of homogeneously high, moderate, or low egg densities. Consideration of the acoustic distributions of adult biomass was also made in the designation of stratum boundaries.

(*ii*) The number of eggs per square metre was rescaled to remove the between-year and within-season variation in mean egg density for each stratum, thus producing a single large set of standardised pilot data from which to estimate optimal allocations and survey statistics. The egg counts at each station were standardised such that their stratum mean was the same over all surveys:

$$y^*_{ijk} = y_{ijk}\frac{\bar{y}_{i..}}{\bar{y}_{ij}}$$

where y^*_{ijk} is the standardised number of eggs per square metre at station k in stratum i in survey j, y_{ijk} is the observed number of eggs per square metre at station k in stratum i in survey j,

$$\bar{y}_{i..} = \frac{1}{A_i n_{ij}} \sum_{j=1}^{A_i} \sum_{k=1}^{n_{ij}} y_{ijk}$$

is the average number of eggs per square metre in stratum i over all surveys that surveyed stratum i, A_i is the number of surveys that included stratum i, n_{ij} is the number of stations in stratum i in survey j, and

$$\bar{y}_{ij} = \frac{1}{n_{ij}} \sum_{k=1}^{n_{ij}} y_{ijk}$$

is the average number of eggs per square metre in stratum i in survey j. Surveys done before the spawning season (9–11 May 1978, 13–17 June 1979) and after it (7–8 October 1978) were not used because their mean egg abundances were zero or very near zero.

(*iii*) The optimum allocation of samples in a stratified random

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Fig. 2. Contours of hoki egg and larval abundance off the South Island west coast from ichthyoplankton surveys in the winters of 1978–1981 and 1987–1988. Surveys done before the spawning season (9–11 May 1978, 13–17 June 1979) and after it (7–8 October 1978) are not shown because their mean egg and larval abundances were zero or very near zero. (Fig. 2 concluded next page.)

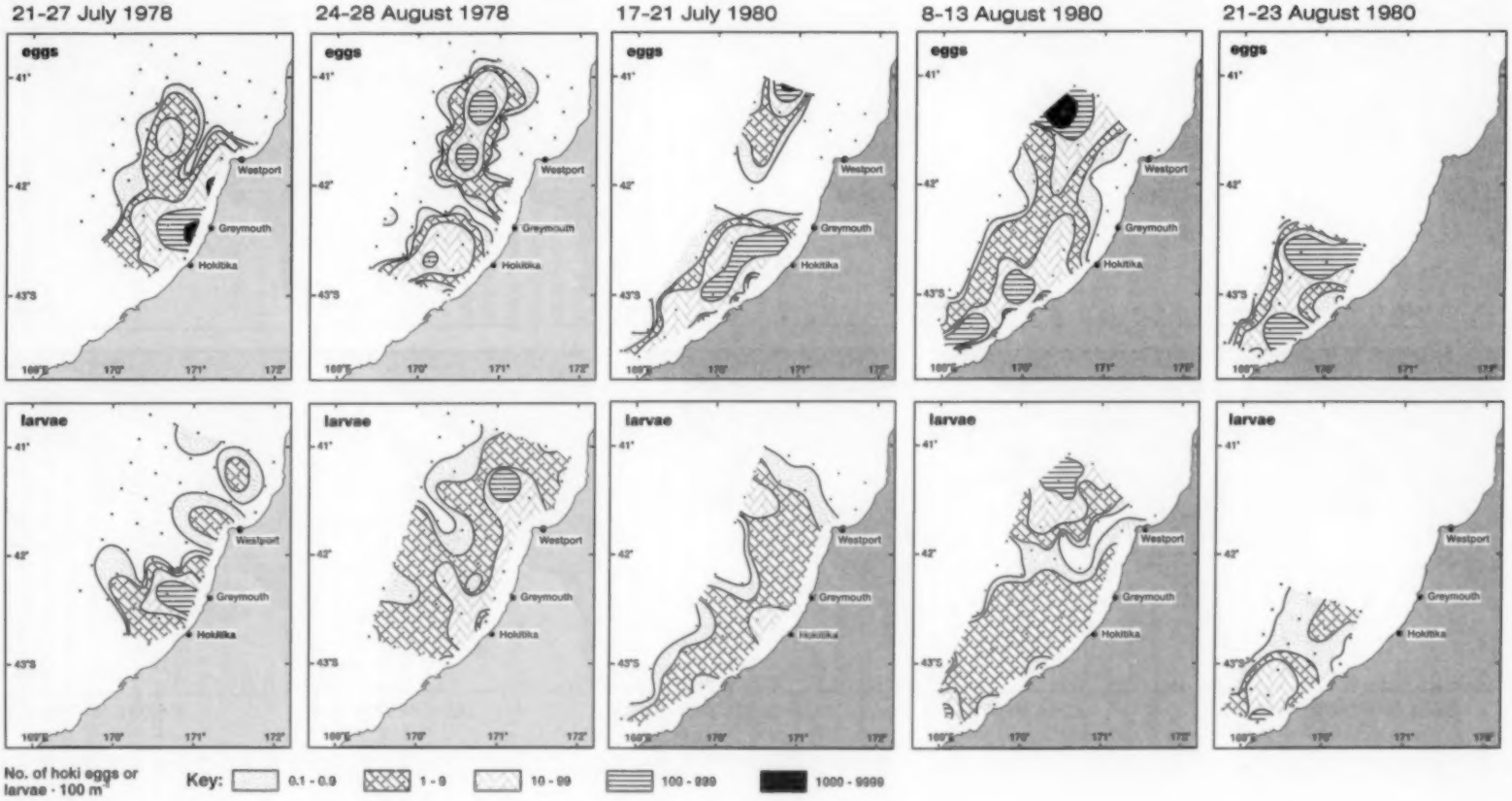

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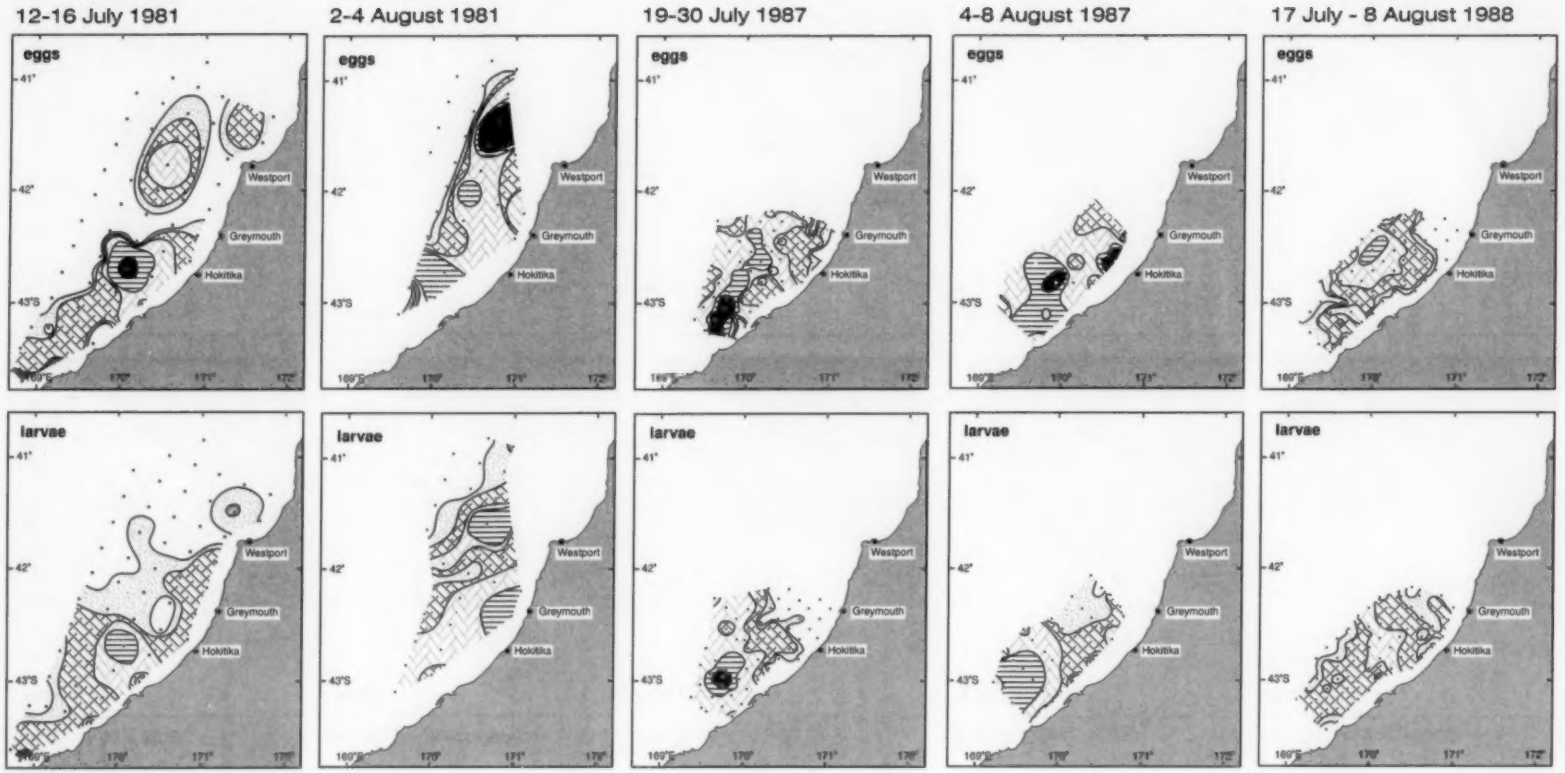
Fig. 2 (*concluded*).



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survey (in terms of obtaining minimum variance for a fixed sample size) is to allocate samples to strata in proportion to the number of units in the stratum (in this case, units are square metres) times the stratum standard deviation (SD) of the values (in this case, values are y^*_{ijk}), assuming that the cost of sampling is constant throughout the survey (Neyman allocation, Cochran 1977, p. 98). However, in this application, allocation was done in proportion to the stratum area times the stratum mean because the means were highly correlated with the strata SDs and probably more reliably estimated than the SDs (Francis 1984). Hence, in a survey of size n, n_i stations were allocated to each stratum such that

$$n_i = n \frac{N_i \bar{y}_{i\cdot}}{\sum_{i=1}^{L} N_i \bar{y}_{i\cdot}}$$

where N_i is the area of stratum i (square metres) and L is the number of strata. A minimum of two stations were allocated to each stratum to allow stratum standard deviations to be calculated in an actual survey.

(*iv*) The stratified mean egg density

$$\bar{y}^*_{st} = \sum_{i=1}^{L} \frac{N_i}{N} \bar{y}_{i\cdot}$$

and the variance of $\bar{y}^*_{st}$

$$V(\bar{y}^*_{st}) = \frac{1}{N^2} \sum_{i=1}^{L} \frac{N_i^2 S_i^{*2}}{n_i}$$

(Cochran 1977, pp. 91–93) were estimated, where N is the total survey area (square metres) and S^*_i is the SD of the standardised data in stratum i. Variances were calculated both using the numbers of stations per stratum from the original data set and for a number of survey sizes, n, allocated to strata using step *iii*. Coefficients of variation (CVs) were derived as $V(\bar{y}^*_{st})^{1/2}/\bar{y}^*_{st}$ for each survey size.

Because the behaviour of the fishing fleet and the distributions of hoki eggs and adults suggested that spawner biomass was higher in the northern area off Westport in August than in July, steps *ii*–*iv* were repeated with the egg data time-stratified between July and August surveys (using survey middates). In this case, the y_{ijk} at each station occupied in July or August were standardised such that their stratum means were the same over all surveys made in July or August. Mean egg density estimates $\bar{y}^*_{st\,Jul}$ and $\bar{y}^*_{st\,Aug}$ were calculated for each month. CVs of the means were derived using the numbers of samples per stratum in the original data set and for a number of survey sizes with samples allocated to strata using step *iii*. Finally, both time- and non-time-stratified CVs were estimated for surveys in which stations were allocated in proportion to N_i only, that is, randomly over the entire Westland area, to compare with the CVs obtained from the Neyman allocation.

Historical biomass

An estimate of the biomass of recruited hoki in the western hoki stock was produced from the historical egg data using the equation

$$(1) \qquad B = \frac{P_0 \times N \times S}{F \times P \times R \times k}$$

where B is biomass of recruited hoki (females ≥65 cm and males ≥60 cm) in the western hoki stock (tonnes), P_0 is the daily egg production (eggs per square metre per day), N is the total survey area (square metres) as above, S is the spawning season length (days), F is the annual fecundity of hoki (eggs per kilogram per year), P is the proportion of mature hoki females in the western stock that goes to Westland to spawn, R is the proportion of the recruited biomass (females plus males) made up by recruited female biomass in the western stock and, $k = 1000$, which converts the result to tonnes.

In the historical biomass calculation, the stratified mean egg density ($\bar{y}^*_{st}$) was estimated by

$$\bar{y}^*_{st} = \frac{N_{Jul}}{N_{JA}} \bar{y}^*_{st\,Jul} + \frac{N_{Aug}}{N_{JA}} \bar{y}^*_{st\,Aug}$$

where N_{Jul} and N_{Aug} are stratum sizes (days) of the July and August time strata, respectively, and $N_{JA} = N_{Jul} + N_{Aug}$. The stratum sizes were 20 days for July and 28 days for August, which were the number of days between the start of the first voyage on 12 July and 31 July and between 1 August and the finish of the last voyage on 28 August, respectively (Fig. 3). These first and last voyages had very low egg densities, consistent with the beginning and end of seasonal spawning. Also, frequencies of ovulated (spawning) ovarian stages in hoki caught in the Westland fishery over three seasons from 1989 to 1991 (Langley 1993) increased from low levels after the first week of July and declined to low levels at the end of August, consistent with the above start and finish dates. The SD of $\bar{y}^*_{st}$ was estimated by

$$\left[\frac{1}{N^2}\left[\frac{N^2_{Jul} S^{*2}_{Jul}}{n_{Jul}} + \frac{N^2_{Aug} S^{*2}_{Aug}}{n_{Aug}}\right]\right]^{1/2}$$

where n_{Jul} and n_{Aug} are the number of samples in the July and August time strata, respectively, in the original data set and S^*_{Jul} and S^*_{Aug} are the SDs of $\bar{y}^*_{st\,Jul}$ and $\bar{y}^*_{st\,Aug}$, respectively.

The stratified mean egg density, $\bar{y}^*_{st}$, was considered an estimate for the density of eggs of all ages combined, on an average day during the hoki egg production season, over all sampled years. This was because the eggs had not been divided into age groups during sorting for the MAF surveys and because the data had been standardised to common stratum means across all surveys and years. The $\bar{y}^*_{st}$ estimate was used to estimate P_0 by using an estimate of Z (instantaneous egg mortality per day). A Z estimate was available from one of the NZOI surveys (17 July to 8 August 1988) which had 61 stations between Hokitika and Cook canyons (Fig. 2). The eggs from this survey were staged and aged using the age-at-stage relationships of Patchell et al. (1987). The Z was estimated as the slope of the log-linear regression of egg density (eggs per cubic metre) versus egg age (days) (r^2 = 0.70). The value of Z was 0.49 with SD = 0.16. The P_0 was estimated using

$$(2) \qquad \bar{y}^*_{st} = \int_{t_f}^{t_h} P_0 e^{-Zt} dt = \frac{P_0}{Z}\left(e^{-Z(t_f)} - e^{-Z(t_h)}\right) = \frac{P_0}{Z}\left(1 - e^{-Zt_h}\right)$$

where t_f and t_h were egg ages at fertilisation (0 days) and hatching (3.33 days), respectively, and Z and embryonic period were both assumed constant. Essentially, the object was to estimate daily egg production, P_0, using the average density of eggs from 3.33 days of production, $\bar{y}^*_{st}$, and the daily mortality rate, Z. This strategy was similar to that used by Lo (1985) to estimate northern anchovy (*Engraulis mordax*) egg production from non-age-structured historical egg data.

Estimates of hoki weight-specific annual fecundity ($F = 2.94 \times 10^5$ eggs·kg^{-1}·year^{-1}, SD = 0.15×10^5; Schofield and Livingston 1998), proportion spawning ($P = 0.77$, SD = 0.02; Vignaux et al. 1995), and female to male recruited biomass proportion ($R = 0.60$, SD = 0.06; N. Bagley, National Institute of Water and Atmospheric Research, unpublished data) were made for the western hoki stock using wide area trawl surveys made out of the spawning season (Sullivan et al. 1995).

Approximate precision of the historical biomass estimate was estimated by generating 500 estimates of biomass using eqs. 2 and 1. The biomass model parameter estimates were obtained from normal distributions formed from the parameter means and SDs given above. No error was assumed for S, and no covariance was assumed between

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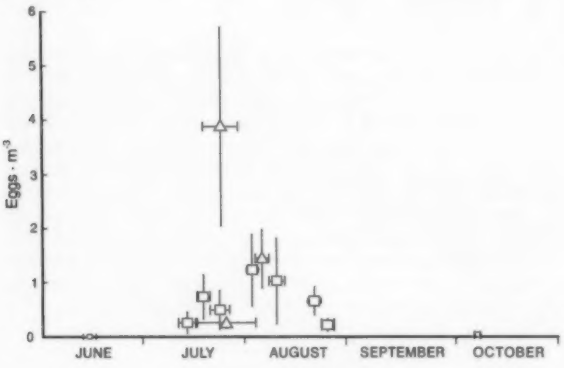
Fig. 3. Mean and 1 SD of density of hoki eggs for each ichthyoplankton survey by date. Horizontal bars represent the start and finish dates of each survey. The survey in May 1978 is not shown. Squares and triangles indicate MAF and NZOI surveys, respectively.



any of the parameters. The CV of the historical estimate was taken as the SD of the 500 estimates divided by their mean.

Results

Ichthyoplankton surveys

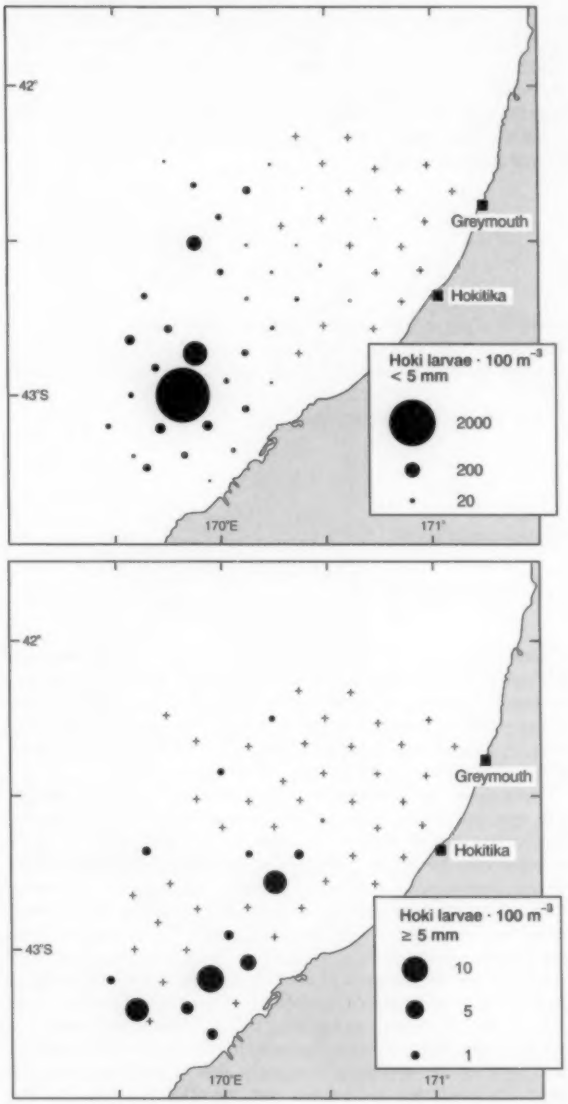
Hoki eggs were most abundant along the shelf edge and slope in most surveys (Fig. 2) between 250 and 750 m (Fig. 1). Regions of high egg abundance were primarily from the Hokitika Canyon area, south to Cook Canyon, and the shelf region off Westport. Exceptions were in July 1978 and August 1987 when high egg numbers occurred in nearshore waters (<250 m) at the head and to the north of Hokitika Canyon (Fig. 2). Egg production appeared to start in early July, peaked in late July to early August, and ended in late August (Fig. 3). Eggs were absent or in very low abundance from Westport to the southern flank of Hokitika Canyon on 5–7 May 1979, 13–17 June 1979, and 7–8 October 1978 (not shown in Fig. 3). In all years of the MAF sampling, higher egg densities were found at the northern stations off Westport in August than in July (Fig. 2). The NZOI sampling in 1987–1988 did not include northern stations.

Hoki larvae generally were abundant where hoki eggs were abundant (Fig. 2), although centres of larval abundance were closer to shore than those of egg abundance for the July and August 1978 surveys and the August 1981 survey. In July 1987, smaller larvae were found further offshore than larger larvae, which were found over the inner shelf (Fig. 4).

Acoustic surveys

The highest densities (tonnes per square kilometre) of adult hoki biomass were detected each year in the southern section of the acoustic survey area (strata 5*a* and 6; Fig. 5) from Hokitika Canyon south to Cook Canyon. In every year, the largest mean biomass (tonnes) in a stratum was in either stratum 5*a* or 6 (Table 1). Strata 1&2 and 4 normally had low transect densities but on occasion contributed large biomass estimates (in excess of 100 000 tonnes and up to 50% of the total biomass estimate of a "snapshot"; Table 1). These large biomass estimates reflected occasional high densities of hoki encountered

Fig. 4. Distribution and abundance of hoki larvae <5 mm standard length (top panel) and ≥5 mm standard length (bottom panel) for the 19–30 July 1987 NZOI survey.



in these strata (although not along the whole of a transect, as was often the case in stratum 5*a*). There was no obvious within-season pattern of biomass changes between these northern strata and the southern ones, but large estimates in the northern strata usually occurred later in the season (late July or August; Table 1). The between-year changes in the mean total biomass also were reflected fairly evenly across strata.

Egg production survey design

Hoki egg abundance (Figs. 2 and 6) and adult hoki biomass (Fig. 5) had similar spatial distributions, showing that egg distributions reflected adult biomass distributions. It was clear (Fig. 6) that egg abundance varied greatly between the

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Fig. 5. Average density of adult hoki for each transect of each acoustic survey. The acoustic survey stratum boundaries are labelled in the first panel. (Fig. 5 concluded next page.)

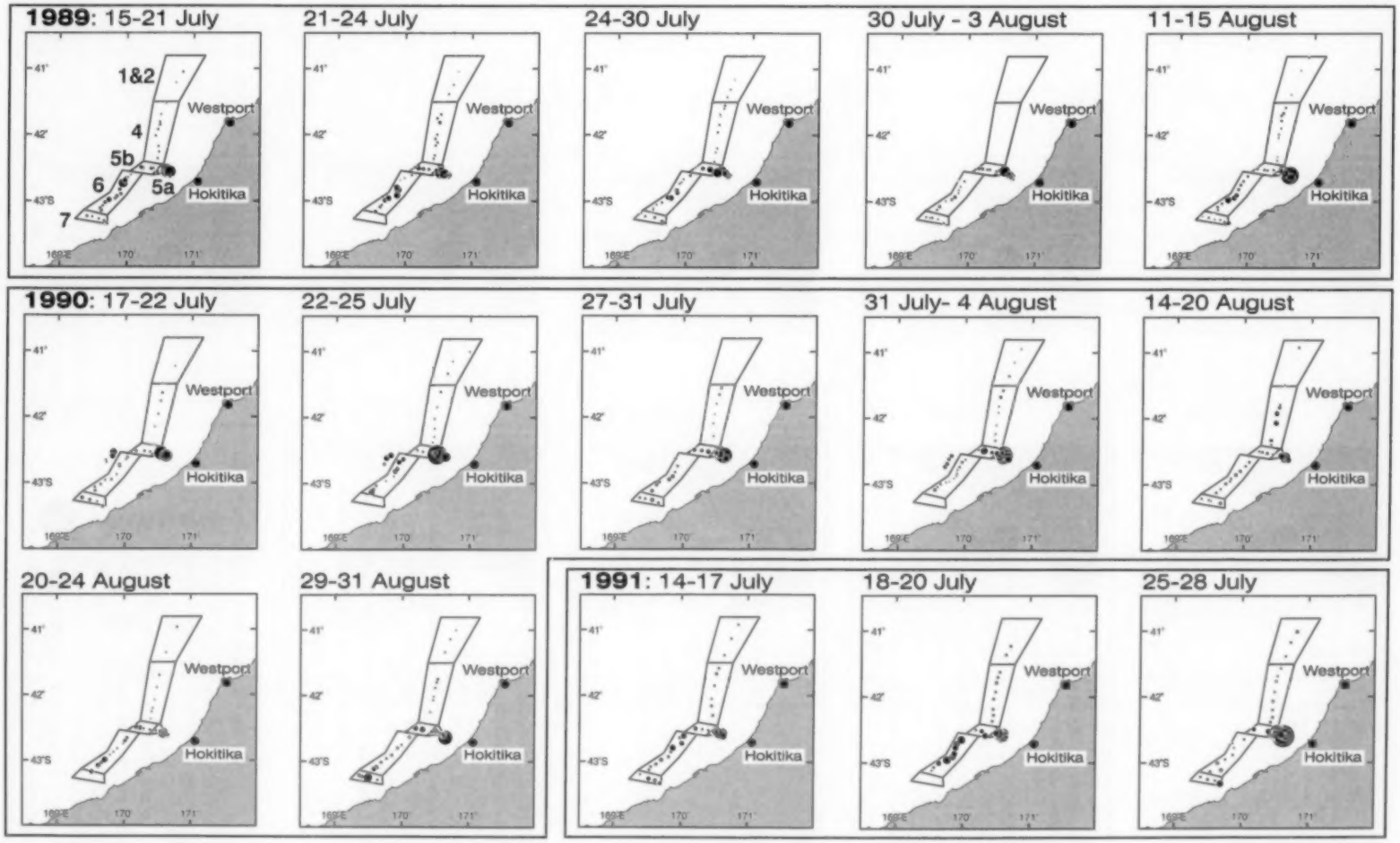

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Fig. 5 (*concluded*).

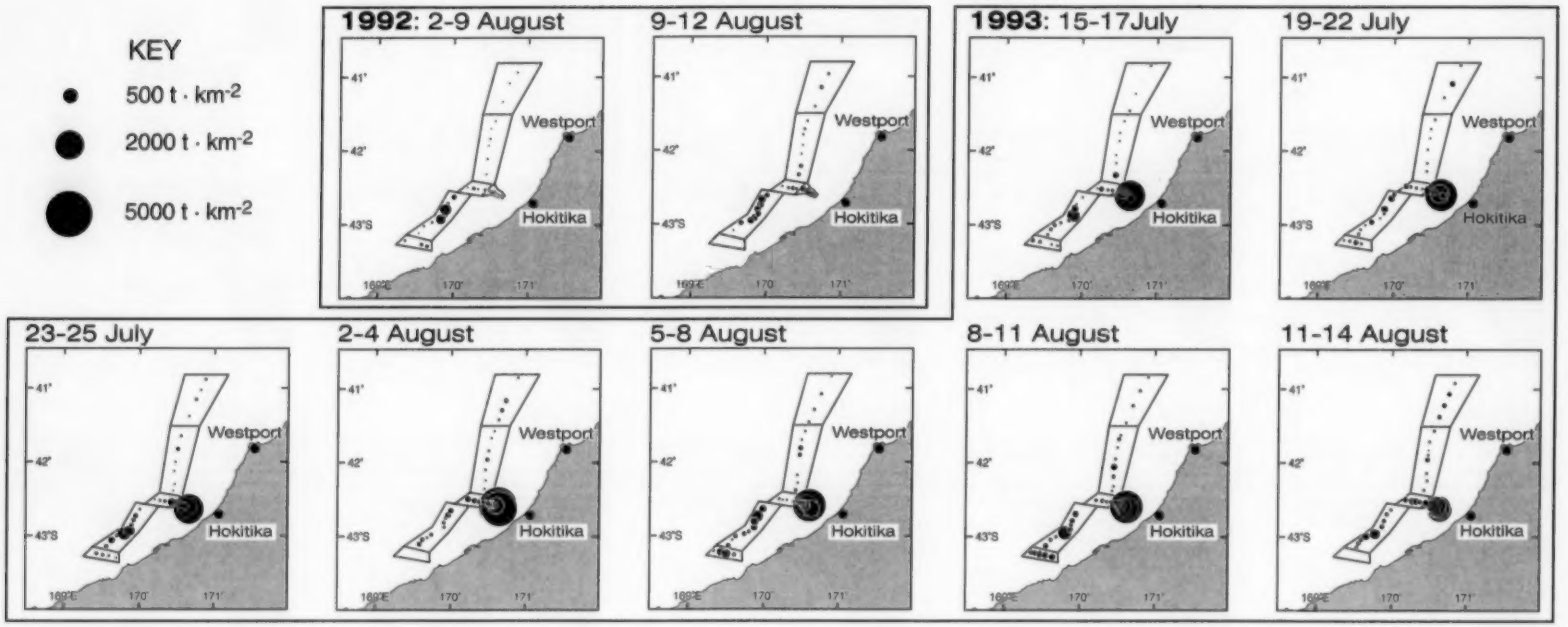

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Table 1. Acoustic estimates of hoki biomass (kilotonnes) for each survey by stratum (Fig. 5).

	Stratum						
Survey date	1&2	4	5*a*	5*b*	6	7	Total
1989							
15–21 July	13	26	66	17	70	6	198
21–24 July	10	38	77	19	101	(6)	250
24–30 July	15	24	36	42	37	7	161
30 July – 3 August	(16)	(29)	43	8	23	4	121
11–15 August	17	33	142	10	54	6	261
Mean	14	30	73	19	57	6	198
1990							
17–22 July	19	29	89	7	26	11	180
22–25 July	16	24	57	5	61	(11)	174
27–31 July	(16)	33	76	23	48	13	209
31 July – 4 August	17	34	65	27	18	(13)	174
14–20 August	38	110	40	9	32	6	234
20–24 August	23	17	18	18	50	(6)	131
29–31 August	20	38	26	27	53	33	197
Mean	21	41	53	17	41	13	186
1991							
14–17 July	31	60	41	12	59	16	219
18–20 July	63	67	56	17	131	16	350
25–28 July	65	52	100	27	25	14	285
Mean	53	60	66	19	72	16	285
1992							
2–9 August	11	19	5	7	138	11	192
9–12 August	71	61	13	11	131	(11)	298
Mean	41	40	9	9	134	11	245
1993							
15–17 July	28	67	77	13	113	12	309
19–22 July	108	36	100	14	64	10	332
23–25 July	47	44	73	22	158	11	355
2–4 August	91	55	169	26	70	(41)	452
5–8 August	59	80	122	5	150	41	457
8–11 August	57	99	126	14	154	37	485
11–14 August	114	71	112	40	101	(37)	475
Mean	72	65	111	19	116	22	410

Note: Estimates in parentheses are for strata that were not sampled during a survey, and were assumed.

designated egg survey space strata and that the $\bar{y}_{i.}$ would contrast greatly between the strata (Table 2). Thus, space-stratified surveys of mean egg abundance were considerably more precise than non-space-stratified surveys (Table 3).

The planktonic and acoustic data also suggested that there was a spatial trend in hoki biomass along the west coast through the spawning season (Fig. 2; Tables 1 and 2), in which egg production rose somewhat later (August) in the northern part of the spawning range than in the southern part, each year. This justified standardising the egg counts for July and August separately (time-stratified) to compare with results when the data were standardised using both months combined (non-time-stratified; Table 2). The CVs using the time-stratified data were considerably smaller than the CVs that were non-time-stratified (Table 3) for both space- and non-space-stratified surveys.

Table 2. Stratum means ($\bar{y}_{i.}$) and standard deviations (SD) of hoki eggs (m^{-2}) derived from the 1978–1988 planktonic data.

	All data		July		August		
Stratum	$\bar{y}_{i.}$	SD	$\bar{y}_{i.}$	SD	$\bar{y}_{i.}$	SD	N_i
A	3	8	6	16	0	0	7181
B	301	722	87	237	531	1081	8626
C	0	0	0	0	0	0	6209
D	54	96	84	166	15	22	6642
E	326	882	386	1161	222	415	10963
F	18	39	16	33	18	44	6303
G	58	68	38	42	83	116	3136

Note: Data were standardised using stratum means from all the data combined, or for July and August separately. Also shown are stratum sizes, N_i ($m^{-2} \times 10^{-6}$). Values for stratum A in August and stratum C were <0.1.

The allocation of station numbers to strata differed considerably between surveys that were time-stratified and those that were not (Table 3). The allocations showed that there should be a large shift in sampling effort to the northern stratum B in August, primarily at the expense of the Hokitika stratum E. Because a minimum of two stations was allocated to each stratum, the numbers of stations allocated did not change in proportion to changes in total station number (Table 3). For strata A and C the allocation remained at two stations as total station number was changed from 75 to 225 for all stratification designs, and for stratum F, the allocation changed from two to three stations only when n reached 175 stations for the non-time-stratified design (not shown). These strata could be omitted from a future survey design, as they consistently contributed very little egg production to the annual total (Table 2). Care would be needed for stratum F, however, which is immediately offshore of stratum E, which had the highest egg production.

Historical biomass

The time- and space-stratified sampling design was used to estimate the historical egg abundance. The estimates of mean egg density on average days during July and August of the hoki egg production season were $\bar{y}^*_{st\,Jul} = 118$ eggs·m^{-2} (CV = 21.4%) and $\bar{y}^*_{st\,Aug} = 153$ eggs·m^{-2} (CV = 24.9%). Season-wide mean egg density (combining estimates for July and August) was $\bar{y}^*_{st} = 138$ eggs·m^{-2} (CV = 17.7%). Season-wide P_0 was 84 eggs·m^{-2}·day^{-1}, with bootstrapped CV = 27.4%. The resulting estimate of historical biomass was 1.48 million tonnes, with bootstrapped CV = 29.4%.

Discussion

Ichthyoplankton and acoustic surveys

The ichthyoplankton and acoustic surveys indicated that hoki spawn primarily around Hokitika Canyon and south along the outer shelf and slope to Cook Canyon. Although large numbers of eggs were caught at some stations over the outer shelf off Westport, the summarised egg abundances (Fig. 6) and the acoustic surveys (Table 1; Fig. 5) showed that adult biomass was lower in this area relative to the Hokitika/Cook Canyon area. For surveys that sampled both southern and northern strata (the MAF surveys), higher egg abundances were found in August than in July at the northern stations off

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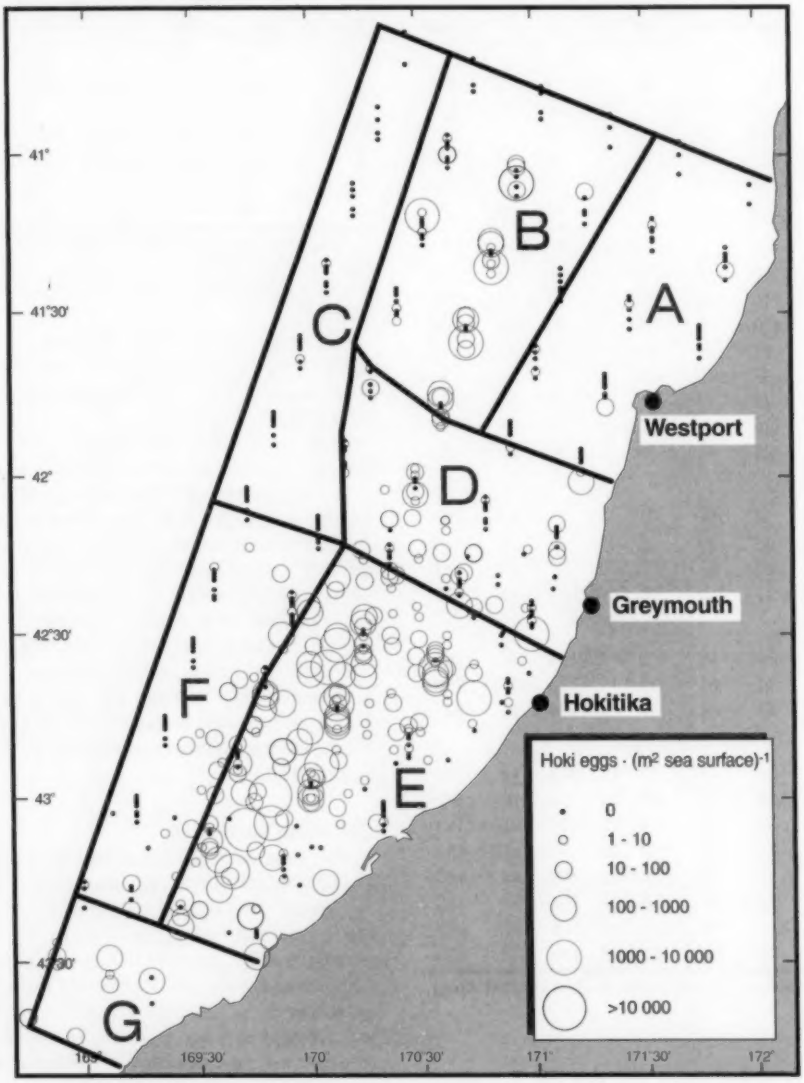
Fig. 6. Hoki egg densities for all stations in the MAF and NZOI surveys used in modelling. Station latitudes were offset slightly to allow individual stations to be seen for each survey and actual station positions are near the centre of each string of station positions. Spatial stratum boundaries used for survey modelling are also shown.



Westport, supporting the hypothesis that adult biomass increases in this northern area in August.

Hoki larvae occurred primarily along the outer shelf and slope. However, larger and presumably older larvae occurred shoreward of the eggs and newly hatched larvae, over the inner shelf. On some surveys, both eggs and larvae were found in relatively high numbers in shallow shelf areas at the head and to the north of Hokitika Canyon (Fig. 2). Because hoki spawn at depths of 400–600 m (Langley 1993), and rarely occur in shallow areas, it is likely that shoreward transport of eggs and larvae from Hokitika Canyon explains these egg and larval distributions. Upwelling at the head of Hokitika Canyon has been observed on a number of occasions (Hall and Vincent 1990; Stanton and Moore 1992; Vincent et al. 1992), and the shoreward advection of hoki eggs and larvae by upwelling has been observed in Cook Strait (Fig. 1), the other major spawning ground of hoki in New Zealand (Murdoch et al. 1990).

Egg production survey design

In this study, projected CVs of Westland hoki egg abundance surveys were estimated using the historical egg survey data. The most precise estimates of abundance were to be found by using surveys that were stratified both in space and in time. Future surveys could be made cheaper and more effective by omitting allocations of stations to strata A and C, and possibly F, and placing those stations into strata with high egg densities and (or) large areas.

Zeldis (1993) suggested that the plankton surveys for

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Table 3. Coefficients of variation (CV) for egg abundance surveys at survey sizes (n) of 75, 150, and 225 stations when time and space stratification were or were not used.

		CV		Allocations						
n		Space-stratified	Non-space-stratified	A	B	C	D	E	F	G
75	July	33.7	54.8	2	9	2	7	51	2	2
	August	23.5	37.5	2	42	2	2	23	2	2
	July and August	18.7	26.8							
	Non-time-stratified	29.0	42.8	2	27	2	4	36	2	2
150	July	23.4	38.8	2	19	2	14	107	3	3
	August	16.3	26.5	2	90	2	2	47	2	5
	July and August	13.2	19.0							
	Non-time-stratified	20.5	30.2	2	56	2	8	76	2	4
225	July	19.0	31.6	2	29	2	21	162	4	5
	August	13.2	21.6	2	135	2	3	72	3	8
	July and August	10.8	15.5							
	Non-time-stratified	16.7	24.7	2	84	2	12	115	4	6

Note: The time-stratified CVs are shown for July and August separately and combined. Also shown are the allocations (stations per stratum) of station numbers to the space strata (A–G) when time-stratification was or was not used.

annual egg production could be interleaved among acoustic "snapshots" to provide an absolute biomass estimate and calibrate acoustic estimates to absolute biomass. Twelve days between starts of each egg survey would allow four 8-day egg surveys and three 4-day acoustic surveys within the 48-day spawning season. We consider that these survey durations would be sufficient to complete egg and acoustic surveys from Westport to Cook Canyon using a space- and time-stratified egg survey design (with 150 stations·survey^{-1}; predicted abundance CV = 13%) and the existing acoustic survey design.

Historical biomass

The estimate of historical biomass (1.48 million tonnes, CV = 29.4%) could be considered to be an estimate of virgin biomass B_0 because most of the historical sampling (the part done by MAF) was done when the Westland hoki fishery was new. However, this estimate is likely to be biased down because the plankton tows were done only from the surface to 200 m, and much of Westland hoki spawning is centred in depths of 400–600 m (Langley 1993). Therefore, the youngest eggs, which had not yet ascended to the sampling depths, would have been undersampled. This would have caused an underestimate of mortality rate that would have biased P_0 downward. The best estimate of B_0 from stock reduction modelling is 2.21 million tonnes (Annala and Sullivan 1997). The historical biomass estimate is 67% of this, but much of this discrepancy could be explained by the likely underestimation of young egg abundance and mortality rate. Also, given the numerous assumptions in the historical biomass calculations, for example that the Z estimate from one egg survey applies equally to all surveys and that S was 48 days, and the assumption that spawning conditions remained stable over the historical period, it is encouraging that the historical estimate is reasonably close to the current B_0 estimate.

In a previous study (Zeldis 1993), simulated CVs of hoki annual egg production estimates were given for a range of egg abundance survey CVs and a range of time intervals between surveys. These simulations were done using the methods of Hauser and Sissenwine (1991), who modelled the uncertainty in estimates of the production of larval fish derived from samples of larval abundance. These hoki annual egg production simulations showed that for an abundance survey CV of 20% and a between-survey interval of 12 days, the predicted CV of the annual egg production estimate was about 10% (see fig. 15 in Zeldis 1993). It is likely that this CV was too optimistic because it was sensitive to factors not explicitly tested in the simulations. Most important among these factors was the effect of random variations in mortality rate of eggs from the assumed constant rate. Hauser and Sissenwine (1991) showed for larvae that the CV of production increased in direct proportion to increase in the CV of mortality rate, so variability in production was quite sensitive to variability in mortality rate. In the current study, the CV of egg abundance input to the bootstrapped estimate of historical biomass precision was 17.7% and the CV of P_0 was 27.4%, so clearly, incorporation of error in Z added substantial variance. So, the CVs of simulated annual egg production estimates in Zeldis (1993) may well have been too low. However, Cordue (1996) has shown that even a relatively imprecise annual egg production survey estimate for hoki biomass (CV = 40%) would yield a great gain (50%) in the "information index" (Cordue 1998) for current and virgin biomass estimates for the Western hoki stock because it would be used as an absolute (rather than relative) biomass estimate in the stock reduction analysis applied in the hoki stock assessment (Annala and Sullivan 1997).

This study used previously unpublished, fishery-independent data (planktonic and acoustic) to describe the spatial and temporal distributions of hoki spawning biomass within the Westland region. The study signified the importance of applying previously collected data as pilot information in the design of resource-intensive egg production surveys. Picquelle and Megrey (1993) also synthesised older data to estimate historical biomasses of walleye pollock (*Theragra chalcogramma*) in Shelikov Strait, Alaska. That study, however, had the advantage of having age-structured egg abundance data and plankton tows that sampled the water column appropriately for quantitative abundance estimates of the target egg species. Lo (1985) synthesised non-age-structured historical northern anchovy egg abundance data with age-structured larval data to estimate historical egg production. In the present study, neither eggs nor larvae were age structured. Nevertheless, the hoki data were exploited to construct an appropriate design for future AEPM

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egg surveys and to ask if such surveys would be likely to be sufficiently precise and accurate for management purposes. Spatial and temporal stratification along the lines used here would be very useful in producing an AEPM biomass estimate sufficiently precise for fisheries management while minimising cost. Also, it appears that a future AEPM survey could monitor biomass accurately if the plankton tows sampled the water column for hoki eggs adequately and the other parameters in the AEPM (Z, S, F, P, R) were accurately estimated.

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Predation by juvenile piscivorous bluefish (*Pomatomus saltatrix*): the influence of prey to predator size ratio and prey type on predator capture success and prey profitability

Frederick S. Scharf, Jeffrey A. Buckel, Francis Juanes, and David O. Conover

Abstract: Capture success, handling time, prey vulnerability, and prey profitability were examined as a function of prey length/predator length ratio for age-0 juvenile bluefish (*Pomatomus saltatrix*) feeding on juvenile striped bass (*Morone saxatilis*) and Atlantic silverside (*Menidia menidia*). For each prey species, bluefish capture success declined linearly and handling time increased exponentially with increasing length ratios between 0.20 and 0.65. However, bluefish capture success was lower and handling times increased at a faster rate when feeding on juvenile striped bass compared with Atlantic silverside. Prey vulnerability, measured as bluefish predation rate, declined exponentially with increasing length ratios for each prey species. Profitability curves were dome shaped for each prey species; however, profitability values and the location of dome peaks differed between prey species. Capture success functions were combined with field length distributions to generate expected frequency distributions of length ratios included in bluefish diets. Comparisons resulted in good agreement between expected and observed sizes in bluefish diets, illustrating the importance of capture success in determining piscivore diets. Our results indicate that, for juvenile fishes, prey-specific morphological and behavioral differences can have significant effects on the outcome of predator–prey interactions and that size-based predation components are best represented as continuous functions of relative fish size.

Résumé : On a examiné le taux de capture, le temps de manipulation, la vulnérabilité des proies et la profitabilité des proies comme fonction du ratio longueur de la proie à longueur du prédateur dans le cas de tassergals (*Pomatomus saltatrix*) juvéniles d'âge 0 se nourrissant de juvéniles de bars rayés (*Morone saxatilis*) et de capucettes (*Menidia menidia*). Pour chaque espèce de proie, le taux de capture du tassergal diminuait linéairement et le temps de manipulation augmentait exponentiellement quand les ratios de longueurs s'accroissaient entre 0,20 et 0,65. Cependant, le taux de capture était plus faible et le temps de manipulation s'accroissait plus rapidement avec des bars rayés juvéniles qu'avec des capucettes comme proies. La vulnérabilité des proies, indiquée par le taux de prédation des tassergals, diminuait exponentiellement quand augmentaient les ratios de longueurs pour chacune des espèces proies. Les courbes de profitabilité étaient en forme de cloche pour chaque espèce proie, mais les valeurs de profitabilité et la position des sommets des courbes différaient d'une espèce proie à l'autre. On a combiné les fonctions de taux de capture avec les distributions des longueurs sur le terrain pour générer des distributions de fréquences attendues des ratios de longueurs dans le régime alimentaire des tassergals. Les comparaisons ont montré qu'il y avait une bonne correspondance entre les tailles attendues et observées des proies composant le régime alimentaire du tassergal, ce qui témoigne de l'importance du taux de capture dans la détermination du régime alimentaire des poissons piscivores. Nos résultats montrent que, pour les poissons juvéniles, les différences morphologiques et comportementales chez les différentes proies peuvent avoir des effets importants sur l'issue des interactions prédateurs–proies, et que les paramètres de la prédation ayant trait à la taille sont le mieux représentés par des fonctions continues de la taille relative des poissons.

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Introduction

Predator–prey interactions during early life history stages have important effects on survival and recruitment processes of fishes (Bailey and Houde 1989). Specifically, size-dependent predation can play a major role in determining the abundance and size structure of prey and predator populations. However, the outcome of size-structured interactions between prey and predator populations that are growing simultaneously is often complex (Werner and Gilliam 1984; Stein et al. 1988; Rice et al. 1993). Therefore, specific components of size-structured predator–prey interactions are typically determined from laboratory experiments using individual predator–prey

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combinations or small groups. Previous work has demonstrated the potential effectiveness of size-dependent information obtained from laboratory predation experiments in predicting the outcome of population-level interactions (Adams and DeAngelis 1987; Rice et al. 1993).

The mechanisms of size-dependent predation processes must be clearly defined to use individual-based laboratory results to aid in predicting the dynamics of fish populations. Individual-based approaches to modelling fish interactions require accurate information on each component of the predation process (Rice et al. 1997). However, laboratory experiments necessary to generate required predation information are often not conducted, but rather, values for species-specific parameters are estimated from best available data or are replaced with those of a similar species. In particular, success or failure of predator capture during individual encounters with prey has often been estimated by using a single discrete threshold value of prey to predator size ratio (Adams and DeAngelis 1987; Madenjian et al. 1991). The use of a discrete value of relative fish size to determine capture success ignores the dynamic nature of size-dependent predation processes. Adams and DeAngelis (1987) indicated that only a 10% change in the value of the capture success parameter resulted in significant differences in mean length of predator populations in their model output.

Several empirical studies have used single predator and prey species to examine size-structured predation on larval (Folkvord and Hunter 1986; Pepin et al. 1992) and juvenile fishes (Rice et al. 1993; Juanes and Conover 1994*a*). Others have examined the vulnerability of single species of larval fishes to a variety of predator types (Bailey and Houde 1989; Cowan et al. 1996; Paradis et al. 1996). However, recent studies examining predation components for multiple prey species are relatively uncommon (Bailey and Batty 1984; Webb 1986; Wahl and Stein 1988), despite the potential importance of prey morphology and behavior in determining prey vulnerabilities and their effect on the outcome of predator–prey interactions (Sih and Moore 1990).

Most research directed towards understanding the effects of size-structured predator–prey interactions on fish populations has focused on the larval period of the life history (Miller et al. 1988; Paradis et al. 1996) whereas the juvenile period has received comparatively less attention (Rice et al. 1993; Wright et al. 1993; Juanes and Conover 1995). Recent evidence indicates that size-related differences in survival rates of juvenile fishes may significantly affect year-class strength (Campana 1996). Therefore, an improved knowledge of the causes of variation in prey and predator abundances may be gained through a more complete understanding of the processes that influence juvenile survival.

The bluefish (*Pomatomus saltatrix*) is an abundant marine piscivorous fish along the Atlantic coast of the United States. Juvenile bluefish have been shown to be important estuarine piscivores during summer months, as their diet consists of a variety of fish species of commercial and recreational importance (Friedland et al. 1988; Juanes et al. 1993). Several studies have demonstrated that juvenile bluefish feed selectively based on prey size, with smaller prey sizes occurring consistently in the diet (Juanes et al. 1994; Juanes and Conover 1995; Scharf et al. 1997). Based on prey choice experiments conducted using Atlantic silverside (*Menidia menidia*) as prey, Juanes and Conover (1994*a*) suggested that juvenile bluefish attack all prey sizes at equal rates, but consume mainly smaller fishes because of higher capture efficiencies.

Here, we determine empirically the rate of capture success, handling time, prey profitability, and prey vulnerability as a function of prey length/predator length ratio for young-of-the-year bluefish feeding on juvenile striped bass (*Morone saxatilis*) and Atlantic silverside. These two prey species frequently occur in the diet of juvenile bluefish in U.S. east coast estuaries and differ considerably in morphology and behavior, with striped bass being a deep-bodied, facultative schooling species with well-developed dorsal and anal fin spines and Atlantic silverside being a slender-bodied, obligate schooling species that lacks well-developed fin spines. We then generate expected frequency distributions of prey to predator size ratios included in bluefish diets using bluefish capture success functions and predator and prey size availability data and compare these distributions with actual prey to predator size ratio distributions occurring in bluefish diets during summer months in two U.S. Atlantic coast estuaries.

Methods

Juvenile bluefish, striped bass, and Atlantic silverside were collected in the Haverstraw Bay region of the lower Hudson River, N.Y. (41°11′N, 73°54′W), during June–August of 1996 by using beach seines. Additional bluefish and Atlantic silverside were collected from Great South Bay, N.Y. (40°10′N, 73°10′W), and Flax Pond, N.Y. (40°57′N, 73°07′W), marine embayments on the south and north shores of Long Island, respectively. Additional striped bass were collected in the lower Hudson River by the New York State Department of Environmental Conservation as part of a juvenile striped bass survey.

Fish were immediately transported to the Flax Pond Marine Laboratory in Old Field, N.Y., and allowed to acclimate for at least 1 week prior to use in experiments. All fish were maintained in tanks with a continuous flow of seawater at ambient temperatures and natural photoperiod throughout the experiments. Because bluefish feeding trials were conducted during a 5-week period in midsummer (10 July to 14 August 1996), water temperatures (20–23°C) and photoperiod (14 h light : 10 h dark) remained relatively constant throughout the experiments.

A total of 53 bluefish ranging from 80 to 155 mm total length (TL) were used in the feeding trials. Bluefish were held in three 800-L holding tanks while being fed a combination of live and previously frozen fish prey throughout the experimental period. Bluefish were sampled randomly from the holding tanks and were mildly anesthetized with tricaine methanesulfonate (MS 222) before being sorted into groups of three to allow for schooling (Juanes and Conover 1994*a*). Individuals were sorted by size to ensure that TL differences between any two members of the group did not exceed 5 mm. Each group of three bluefish was then placed in a 200-L tank and starved for 24 h to standardize hunger levels across feeding trials. Each group of three bluefish was then placed in one of four 215-L experimental chambers, each equipped with a transparent plexiglass viewing window, and allowed to acclimate for at least 8 h prior to the initiation of a feeding trial.

Each feeding trial consisted of a group of three bluefish presented with a group of 10 striped bass or 10 Atlantic silverside with TL differences not exceeding 2.5 mm between any two prey fish in a group. Juvenile striped bass prey ranged in size from 25 to 80 mm TL whereas Atlantic silverside ranged from 20 to 85 mm TL. Throughout the 5-week experimental period, various combinations of prey and predator length groups were used to generate a continuous range of prey length/predator length ratios from 0.20 to 0.65 for each prey

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species (Table 1), which represents a natural range of length ratios occurring in the field (Scharf et al. 1997). Both large and small bluefish were used to obtain prey length/predator length ratios at each end of this range for each prey species. Prey were added to chambers already containing bluefish using a hollow, transparent plexiglass cylinder that was placed vertically into the chamber. Prey were placed within the cylinder and allowed to acclimate for at least 5 min before the cylinder was removed and bluefish had access to the prey. Experimental chambers were illuminated during feeding trials using a 150-W halogen light positioned about 1 m above each chamber. Each 15-min feeding trial was recorded using a standard Hi-8 video camera mounted about 1 m from the viewing window. After each trial, all live prey fishes were counted and removed and bluefish were returned to the 800-L holding tanks.

Throughout the experimental period, bluefish were sampled randomly with replacement from the holding tanks. This was done to avoid the effects of potential learned behavior by the bluefish resulting from continuous holding in the experimental chambers for extended periods. Our experimental procedure for random sampling of bluefish from the holding tanks, placement into tanks for a 24-h starvation period, and subsequent placement into experimental chambers ensured that once used in a feeding trial, bluefish of a given group could not be used during the next two trials. This translated into a minimum time between use in feeding trials of 36 h for any given bluefish. Given the high consumption and gut evacuation rates exhibited by juvenile bluefish during estuarine residency (Juanes and Conover 1994*b*; Buckel et al. 1995), we felt that our experimental protocol was sufficient to minimize potential predator learning while maintaining adequate hunger levels.

Capture success was calculated as the proportion of bluefish capture attempts that resulted in prey consumption. A capture attempt was defined as a directed strike at a prey fish, wherein a single bluefish oriented toward an individual prey fish and exhibited a burst in swimming speed culminating in a lunge in an attempt to grasp the given prey fish. Handling time, defined as the time from capture of a given prey fish until swallowing activity ceased, was estimated to within 0.03 s for each successful capture. The vulnerability of each prey species to bluefish predation was assessed by plotting predation rate as a function of prey length/predator length ratio. Predation rate was calculated as the percentage of prey offered that was eaten per number of predators per unit time (% prey eaten × no. predators^{-1} × hours^{-1}).

For each prey species, relationships between capture success and relative fish size were analyzed by using least squares regressions (StataCorp. 1995). Handling times and prey vulnerabilities were linearized by using logarithmic transformations and analyzed as a function of prey length/predator length by using least squares regressions. Least squares regressions were compared by using analysis of covariance (Sokal and Rohlf 1995). Nonlinear least squares regression models fit to the exponential relationship $y = ae^{bx}$ (StataCorp. 1995) are presented for handling time and prey vulnerability relationships because back-transformation of linearized functions is subject to bias (Beauchamp and Olsen 1973). Means (± 1 SE) were calculated at each 0.05 interval of prey length/predator length ratio for bluefish capture success, handling time, and prey vulnerability and are illustrated in the figures for simplification. We wanted to ensure that the prey to predator size ratio, and not predator size alone, was the variable responsible for our results. To accomplish this goal, residuals of linear regressions for bluefish capture success, handling time, and prey vulnerability were regressed against bluefish TL to examine the relationship between predation components and bluefish body size across the range of predator lengths used in the experiments.

Profitability was estimated for each prey species as a function of prey length/predator length by combining functions derived from the feeding trials for relative prey mass ingested, handling time, and capture success (prey mass/predator mass × handling time^{-1} × capture probability). To examine the influence of capture success, profitability was also estimated with capture success functions removed from the equations.

Table 1. Number of bluefish feeding trials conducted at each interval of prey length/predator length ratio using striped bass and Atlantic silverside as prey.

Prey length/predator length ratio	Striped bass	Atlantic silverside
0.200–0.249	4	3
0.250–0.299	4	4
0.300–0.349	7	7
0.350–0.399	6	5
0.400–0.449	7	7
0.450–0.499	4	5
0.500–0.549	5	5
0.550–0.599	4	3
0.600–0.649	0	3
Total	41	42

For each prey species, bluefish and prey length distributions observed in the field from early July to mid-August were combined to generate a probability distribution of prey length/predator length ratios occurring in the field during this time period. Prey and predator sizes were obtained for this time period to ensure that size distributions of predator and prey coincided with those used in the laboratory experiments. Bluefish capture success functions were combined with each probability distribution to generate an expected frequency distribution of prey length/predator length ratios included in bluefish diets for each prey species. Expected frequency distributions were generated for Atlantic silverside using predator and prey length distributions occurring in Great South Bay, N.Y., on four dates from 5 July to 9 August in 1989 and for juvenile striped bass using length distributions occurring in the lower Hudson River, N.Y., on five dates from 7 July to 4 August in 1993. Expected frequency distributions were compared with actual frequency distributions of length ratios consumed by bluefish in the field at the same sampling locations on the dates indicated by using chi-square analysis.

Results

A total of 83 bluefish feeding trials were conducted that generated 485 observed attacks resulting in 261 successful prey captures. Bluefish capture success declined linearly with increasing prey length/predator length ratio when feeding on each prey species (striped bass: $y = 1.166 - 1.926x$, $p < 0.001$, $r^2 = 0.41$; Atlantic silverside: $y = 1.245 - 1.419x$, $p < 0.001$, $r^2 = 0.42$) (Fig. 1). Regression slopes were not statistically different ($F_{1,72} = 0.591$, $p > 0.25$); however, elevation of Atlantic silverside capture success was significantly higher than that of striped bass ($F_{1,75} = 30.719$, $p < 0.001$). There was no significant linear relationship between residuals of capture success versus prey length/predator length regressions and bluefish total length for either prey species (striped bass: $p = 0.406$; Atlantic silverside: $p = 0.721$).

Bluefish handling times increased exponentially with increasing prey length/predator length ratio for each prey species (striped bass: $\ln(y) = 0.112 + 7.108x$, $p < 0.001$, $r^2 = 0.54$; Atlantic silverside: $\ln(y) = 0.588 + 3.933x$, $p < 0.001$, $r^2 = 0.41$) (Fig. 2). Regression slopes were significantly different, with bluefish handling time increasing at a faster rate when feeding on striped bass ($F_{1,239} = 7.968$, $p < 0.005$). There was no significant linear relationship between residuals of handling time

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Fig. 1. Proportion of successful captures as a function of prey length/predator length ratio for juvenile bluefish feeding on striped bass (●) and Atlantic silverside (▲). Data points represent means ±1 SE. Lines represent linear regression equations presented in the text.

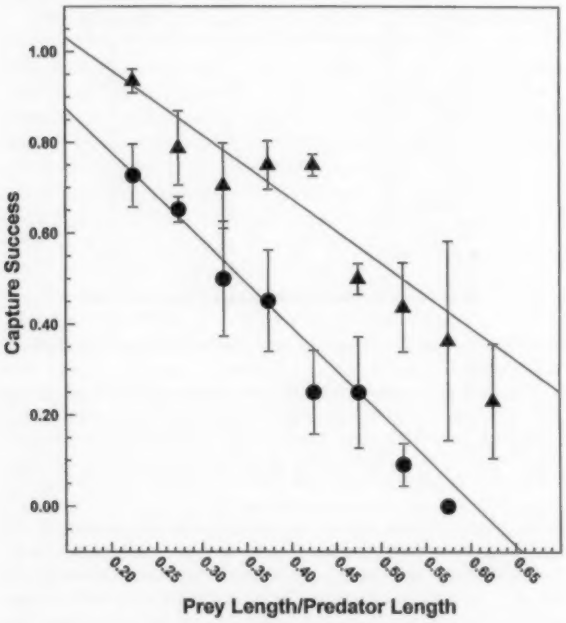
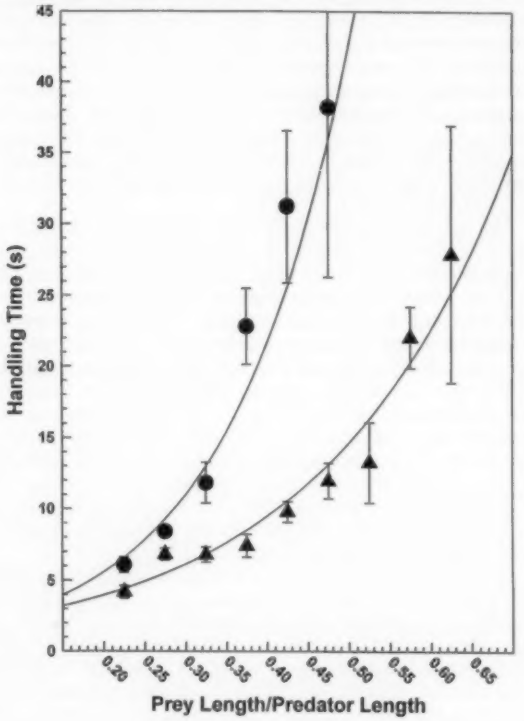


Fig. 2. Bluefish handling time as a function of prey length/predator length ratio for bluefish feeding on striped bass (●) and Atlantic silverside (▲). Data points represent means ±1 SE. Curves represent nonlinear regression equations presented in the text.



versus prey length/predator length regressions and bluefish total length for either prey species (striped bass: $p = 0.618$; Atlantic silverside: $p = 0.156$). For future predictive purposes, unbiased nonlinear least squares regression models were generated for bluefish handling time feeding on each prey species (striped bass: $y = 1.442e^{6.768x}$, $p < 0.001$, $r^2 = 0.78$; Atlantic silverside: $y = 1.671e^{4.342x}$, $p < 0.001$, $r^2 = 0.83$).

Vulnerability of striped bass to bluefish predation declined with increasing prey length/predator length ratios ($\ln(y + 1) = 0.812 - 1.384x$, $p < 0.001$, $r^2 = 0.30$). Atlantic silverside vulnerability also declined with increasing relative prey size ($\ln(y + 1) = 1.373 - 2.061x$, $p < 0.001$, $r^2 = 0.47$) (Fig. 3). Slopes of vulnerability equations were not significantly different between prey species ($F_{1,76} = 0.922$, $p > 0.25$). Elevations were significantly different ($F_{1,79} = 26.719$, $p < 0.001$), with Atlantic silverside being more vulnerable to bluefish predation across the range of length ratios used in the experiments. There was no significant linear relationship between residuals of predation rate versus prey length/predator length regressions and bluefish total length for either prey species (striped bass: $p = 0.873$; Atlantic silverside: $p = 0.921$). For future predictive purposes, unbiased nonlinear least squares regression models were generated for prey vulnerabilities (striped bass: $y = 2.375e^{-5.035x}$, $p < 0.05$, $r^2 = 0.44$; Atlantic silverside: $y = 11.598e^{-7.200x}$, $p < 0.001$, $r^2 = 0.72$).

Profitability as a function of prey length/predator length ratio was calculated using the capture success and handling time functions given above, as well as functions describing the relationship between prey mass/predator mass and prey length/predator length for each prey species (striped bass: $y = 1.686x^{3.280}$; Atlantic silverside: $y = 0.854x^{3.072}$). Profitability curves calculated both with and without capture success functions were dome shaped for each prey species (Fig. 4). However, profitability estimates incorporating capture success were much lower, extended over a narrower range of length ratios, and had peaks at lower length ratios than estimates that did not incorporate capture success. Striped bass profitability peaked at a length ratio of about 0.30 (Fig. 4*a*), much lower than Atlantic silverside profitability, which peaked at a length ratio of about 0.40 (Fig. 4*b*).

Probability distributions of prey length/predator length ratios occurring in the field peaked at ratios between 0.40 and 0.45 for striped bass as prey and at ratios between 0.20 and 0.25 for Atlantic silverside as prey during midsummer of 1993 and 1989, respectively (Figs. 5*a* and 6*a*). For both prey species, multiplying the probability distributions of length ratios occurring in the field by species-specific capture success functions yielded higher relative frequencies at lower prey length/predator ratios in the resulting expected frequency distributions (Figs. 5*b* and 6*b*). Chi-square analysis did produce a significant difference between expected and actual (Fig. 5*c*) distributions of length ratios included in bluefish diets for striped bass as prey ($\chi^2_9 = 64.500$, $p < 0.001$). Similar analyses did not result in a significant difference between expected and actual (Fig. 6*c*) distributions of length ratios included in bluefish diets for Atlantic silverside as prey ($\chi^2_{16} = 10.142$, $0.75 < p < 0.90$).

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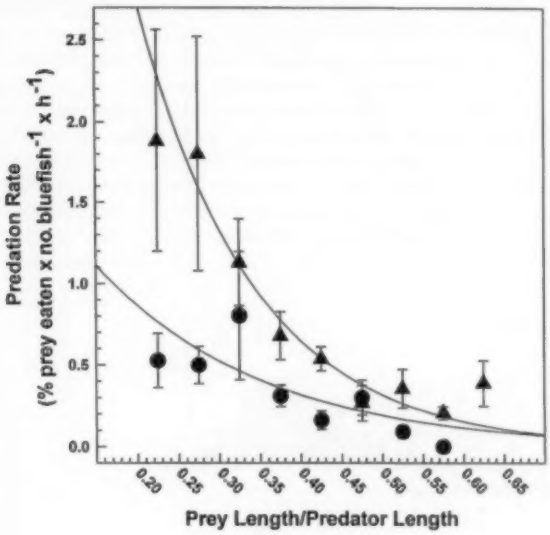
Fig. 3. Bluefish predation rate on striped bass (●) and Atlantic silverside (▲) as a function of prey length/predator length ratio. Data points represent means ±1 SE. Curves represent nonlinear regression equations presented in the text.



Discussion

Capture success, handling time, and prey vulnerability

In our study, juvenile bluefish capture success declined with increasing prey length/predator length ratios for juvenile striped bass and Atlantic silverside. Juanes and Conover (1994*a*) also observed a decline in juvenile bluefish capture success with increased size of Atlantic silverside prey. Similarly, Heatherly (1993) found that capture success of juvenile bluefish feeding on spot (*Leiostomus xanthurus*) declined with increases in prey length/predator length ratios for a range of length ratios similar to that used here. Previous studies have demonstrated a strong negative relationship between piscivore capture success and prey size for several taxa (Miller et al. 1988; Litvak and Leggett 1992). However, these studies focused on experiments conducted using larval fishes as prey. Capture success information for juvenile prey fishes representing several taxa is not available.

For the range of prey length/predator length ratios examined here, juvenile bluefish had higher capture success on Atlantic silverside compared with striped bass. Bailey and Batty (1984) found differences in capture success of medusae predators feeding on four species of larval fishes. They suggested that observed differences in predator capture efficiency resulted from prey-specific differences in reactivity to encounters with predators. For piscivorous fish predators, both increased prey body depth and the presence of spines have been shown to negatively affect predator capture efficiencies (Hoogland et al. 1956; Webb 1986; Wahl and Stein 1988). Our results support previous findings that reveal the potential importance of prey morphology and behavior in affecting the outcome of predator–prey encounters. Video observations indicate that considerable morphological differences between striped bass and Atlantic silverside combined with earlier reactions to bluefish attacks by striped bass produced the observed differences in bluefish capture success.

Bluefish handling time increased exponentially with increasing prey length/predator length ratio for both prey species. These results are consistent with previous studies in demonstrating the link between predator and prey size and the time needed to manipulate and ingest prey (Werner 1974; Mittelbach 1981; Juanes and Conover 1994*a*). Significant differences occurred in the slopes of the handling time curves between prey species, with bluefish handling time accelerating at a faster rate for striped bass than for Atlantic silverside. Largemouth bass (*Micropterus salmoides*) handling time was also found to vary among prey types with contrasting morphologies (Hoyle and Keast 1987). Among fish prey, Hoyle and Keast (1987) observed the fastest rate of increase in handling time for prey with the greatest body depth relative to length. Our findings agree with this result.

Vulnerability of juvenile striped bass and Atlantic silverside to bluefish predation declined exponentially with increasing prey length/predator length ratios, suggesting that the smallest prey sizes were most susceptible. In a review of the effects of body size on larval fish vulnerability, Paradis et al. (1996) show that larval vulnerability to predation by fishes is dome shaped, with maximum vulnerability occurring at prey length/predator length ratios of about 10%. Researchers have argued that dome-shaped vulnerability of larval fishes may arise because, as prey size increases, probabilities of predator encounter and (or) attack increase, while larval susceptibility to predator capture declines (Litvak and Leggett 1992; Pepin et al. 1992). For juvenile fishes, our results indicate that, relative to predator size, the smallest prey sizes are most vulnerable to predation by piscivorous fishes. Except for only the largest prey length/predator length ratios, the range of length ratios examined in this paper extends over the entire range of length ratios occurring in the field during midsummer months for bluefish feeding on these two prey species. Unlike interactions between larval fish prey and fish predators, wherein prey length/predator length ratios are typically less than 20% (Miller et al. 1988; Paradis et al. 1996), prey length/predator length ratios for interactions between juvenile fish prey and fish predators are usually higher than 20% (Rice et al. 1993), ranging from 15 to 85% for bluefish and their prey in our study. Based on our results, vulnerability curves of juvenile fishes to fish predators may peak at the smallest prey length/predator length ratios and decline continuously thereafter, which may simply represent a continuation of the vulnerability curves of larval fishes to fish predators similar to those presented by Paradis et al. (1996).

Prey profitability and bluefish diet

Previous work directed at predicting predator diet using foraging theory suggests that even subtle differences in prey behavior and morphology can dramatically affect foraging relationships (Werner and Gilliam 1984; Sih and Moore 1990). For example, Wahl and Stein (1989) found that differences in taxon-specific movement patterns among esocids led to different levels of vulnerability to largemouth bass predation. We found prey-specific differences in profitability estimates and vulnerability to juvenile bluefish predation. Morphological differences resulted in striped bass providing greater benefits in terms of prey mass ingested relative to Atlantic silverside

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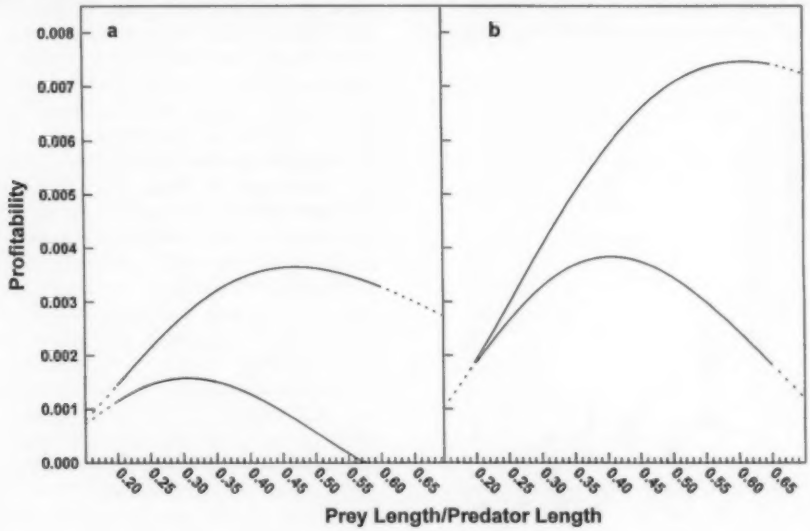
Fig. 4. Prey profitability as a function of prey length/predator length ratio for (*a*) striped bass and (*b*) Atlantic silverside. Upper profitability curves calculated as (prey mass/predator mass × handling time^{-1}); lower profitability curves calculated as (prey mass/predator mass × handling time^{-1} × % capture success). Solid line indicates the range of prey length/predator length ratios examined in this study.



of the same length. However, the increased mass per unit length of striped bass combined with prey behavioral differences in escape tactics also induced a much greater cost in terms of lower capture efficiency and increased handling time. The availability of prey with disparate levels of vulnerability to predation may have important effects on predator foraging success and prey survival.

Foraging theory has been developed extensively for planktivorous fishes and predicts that predators should include prey in the diet based on their ratio of net energy gain to handling time cost relative to other prey (Mittlebach 1981; Stephens and Krebs 1986). However, various authors have hypothesized that capture success is more important for piscivores than for planktivores and may be a critical determinant of piscivore diet (Breck 1993; Juanes 1994; Juanes and Conover 1994*a*). The inclusion of capture success functions in our calculation of profitability for each prey had significant effects on relative profitabilities. In addition to the expected lowering of profitabilities for each prey, the incorporation of capture success changed the relative values of profitability between prey species, with Atlantic silverside becoming even more profitable compared with striped bass. More importantly, profitability curves that included capture success extended over a narrower, and likely more realistic, range of prey length/predator length ratios and peaked at lower length ratios compared with curves without capture success.

Relative frequency distributions of prey length/predator length ratios were generated from length ratios occurring in the field and laboratory-derived capture success functions. These predicted distributions showed good agreement when compared with distributions of prey length/predator length ratios actually consumed by juvenile bluefish in midsummer. Although a significant difference was detected between expected and observed length ratio distributions for bluefish feeding on juvenile striped bass, the peak of the expected frequency distribution was only 5% higher on the prey length/predator length axis than the peak in the distribution of length ratios actually consumed. Our results indicate that estimates of the size composition of prey in bluefish diets may be obtainable from prey and predator length frequencies in the field and simple capture success functions. These findings support previous research on the importance of size-dependent capture success in determining the sizes of prey included in bluefish diets. For prey length/predator length ratios between about 0.15 and 0.60, Juanes and Conover (1994*a*) concluded that bluefish attack rates on Atlantic silverside are independent of prey size and suggest that observed size-selective feeding patterns of juvenile bluefish may result from differential size-based capture success rather than active predator choice. The influence of capture success on our estimates of prey profitability and the close match between predicted and observed distributions of length ratios in bluefish diets indicate that capture success is an important component of piscivore feeding and may be largely responsible for apparent predator selectivities. It is important to note that for diet predictions to be accurate, the size ranges of predator and prey used to estimate encounter and capture probabilities must closely approximate those occurring naturally in the environment. In our study, experiments were conducted using naturally occurring sizes of bluefish and prey during midsummer in U.S. east coast estuaries, and our comparisons with sizes of prey actually consumed in the field were done using only bluefish and prey captured during this time of the year.

Foraging models and piscivores

Important advances have been made towards the understanding of size-structured species interactions and their role in population-level processes and community dynamics (Werner and Gilliam 1984; Ebenman and Persson 1988; Stein et al. 1988). With the advent of individual-based modelling

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Fig. 5. Relative frequency distributions of prey length/predator length ratios for bluefish feeding on juvenile striped bass. Distribution *a* represents the relative probability of encounter for specific prey length/predator length ratios during 7 July to 4 August of 1993. Distribution *b* is generated through the multiplication of distribution *a* with the laboratory-derived capture success function for bluefish feeding on juvenile striped bass and represents the expected relative frequency distribution of prey length/predator length ratios occurring in bluefish diets during this time period based only on encounter and capture probabilities. Distribution *c* represents the relative frequency distribution of prey length/predator length ratios that were actually observed in the diets of bluefish feeding on striped bass during this time period. Chi-square statistics are presented for the comparison between distributions *b* and *c*.

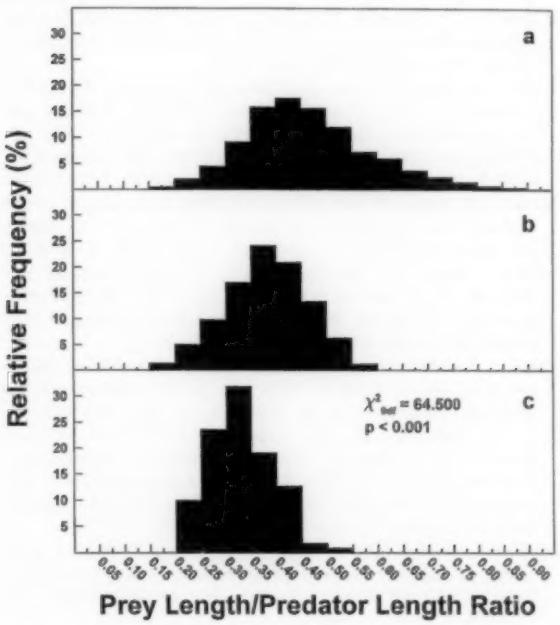
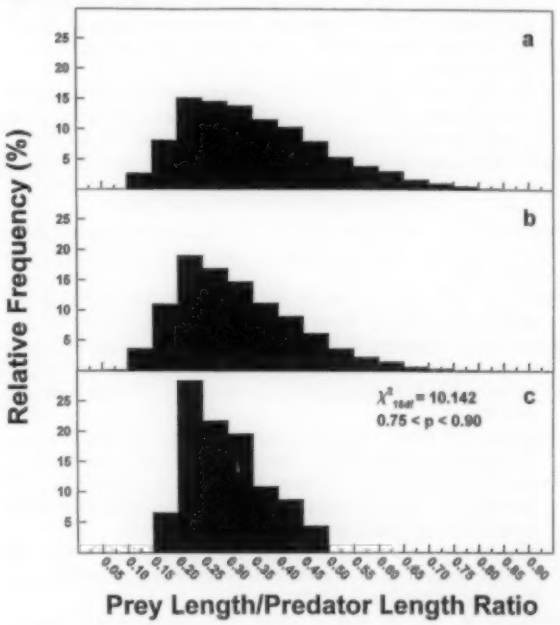


Fig. 6. Relative frequency distributions of prey length/predator length ratios for bluefish feeding on Atlantic silverside. Distribution *a* represents the relative probability of encounter for specific prey length/predator length ratios during 5 July to 9 August of 1989. Distribution *b* is generated through the multiplication of distribution *a* with the laboratory-derived capture success function for bluefish feeding on Atlantic silverside and represents the expected relative frequency distribution of prey length/predator length ratios occurring in bluefish diets during this time period based only on encounter and capture probabilities. Distribution *c* represents the relative frequency distribution of prey length/predator length ratios that were actually observed in the diets of bluefish feeding on Atlantic silverside during this time period. Chi-square statistics are presented for the comparison between distributions *b* and *c*.



techniques, our ability to incorporate specific information on size-based interactions into foraging models has increased markedly (DeAngelis and Gross 1992; Van Winkle et al. 1993). However, the modelling of complex species interactions requires extensive size-specific data. Individual-based modelling approaches have often assumed threshold values of prey size/predator size ratios to determine capture of juvenile fishes given an encounter with a piscivore, with prey size/predator size ratios below threshold values resulting in 100% capture efficiency and those above always resulting in failed capture attempts (Adams and DeAngelis 1987; Madenjian et al. 1991). Our data suggest that using threshold values for capture success may not be realistic. This is particularly important, given that model outputs can be extremely sensitive to variation in this parameter (Adams and DeAngelis 1987). Cowan et al. (1996) proposed that differences in predator attributes such as swimming speed and search pattern may result in differential encounter rates with larval prey fishes, with considerable effects on larval survival rates. For juvenile prey fishes, differences in prey morphology and behavior may strongly affect predator success rates and, hence, prey profitabilities and vulnerabilities over a range of relative fish sizes. Therefore, the outcomes of predator–prey interactions among juvenile fishes are likely to be species specific, with predation components best represented as continuous functions of relative prey and predator sizes such as those used by Rice et al. (1993). Our findings for bluefish suggest that the inclusion of specific sizes of prey fishes in the diets of pisicivorous fish predators may be estimated with only knowledge of encounter and capture probabilities based on relative body size. The use of accurate field and laboratory information when estimating these parameters for foraging models should lead to more reliable predictions of size-based interactions.

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Assemblages and biogeography of demersal fishes of the east coast of North America

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Abstract: This study describes and maps demersal fish assemblages for the east coast of North America from Cape Hatteras, North Carolina, to Cape Chidley, Labrador, evaluates evidence for interannual shifts in assemblage distribution, and examines the relationship of the assemblages to accepted biogeographical boundaries. Demersal trawl survey data collected from 1975 to 1994 were analyzed. Visual classification of distribution maps for the 108 most abundant demersal species revealed nine species groups, based on both geography and depth distribution. Eighteen assemblage groups were identified using principal components analysis (PCA) and mapped. Assemblage groups were also identified by cluster analysis. Fish assemblages identified by both methods were spatially coherent. Assemblage distribution patterns were not consistent with accepted biogeographical boundaries. The PCA explained only 56.3% of the variance in distribution of the species, indicating that the assemblages should be interpreted as indeterminate, potentially adaptable entities rather than as rigid ecological constructs. Assemblages were persistent in composition through time but appeared to shift in location. The apparent looseness of the assemblages and their persistence through time in spite of severe impacts from fishing suggest that single-species management approaches may not be entirely inappropriate for the major groundfish species in the study area.

Résumé : Nous avons caractérisé les assemblages de poissons benthiques de la côte est de l'Amérique du Nord, depuis le cap Hatteras, en Caroline du Nord, jusqu'au Cap Chidley, au Labrador, pour en faire la description et produire une représentation cartographique de leur distribution, évaluer les signes dénotant des changements de distribution survenant au cours d'une même année et examiner la relation entre les assemblages et les limites biogéographiques qui leur sont généralement attribuées. Nous avons analysé les données obtenues par des relevés au chalut de fond de 1975 à 1994. D'après une classification visuelle fondée sur les cartes de distribution des 108 espèces benthiques les plus abondantes, nous avons distingué neuf groupes d'espèces en fonction de leur distribution géographique et de leur distribution en profondeur. L'analyse des composantes principales (ACP) a permis de définir 18 groupes d'assemblages dont nous avons établi la cartographie. Nous avons aussi défini des groupes d'assemblage par analyse typologique. Les premiers assemblages définis par les deux méthodes étaient spatialement cohérents. Par ailleurs, nous avons constaté que la distribution des assemblages ne concordait pas avec les limites biogéographiques qui leur sont habituellement attribuées. Les résultats de l'ACP n'expliquaient que 56,3 % de la variance de la distribution des espèces, ce qui pourrait signifier qu'il y a lieu de considérer les assemblages comme des entités indéterminées et susceptibles de s'adapter, au lieu de groupes écologiques invariables. Les assemblages ont gardé la même composition, mais leur emplacement semblait changer. Vu l'apparente mobilité et la pérennité que présentent les assemblages malgré le lourd impact de la pêche, il se pourrait qu'un mode de gestion où les espèces sont considérées individuellement ne soit pas entièrement injustifié pour les principales espèces de fond qu'on trouve dans la zone étudiée.

[Traduit par la Rédaction]

Introduction

There have been several previous studies of demersal fish assemblages on parts of the continental shelf off the east coast of North America (Colvocoresses and Musick 1984; Mahon and Sandeman 1985; Overholtz and Tyler 1985; Mahon and Smith 1989*a*; Gabriel 1992; Gomes et al. 1992, 1995; Gomes 1993; Guadalupe Villagarcia 1995), although few have examined the northern Labrador Shelf. One other study examined the entire region using 1970–1980 trawl survey data from Cape

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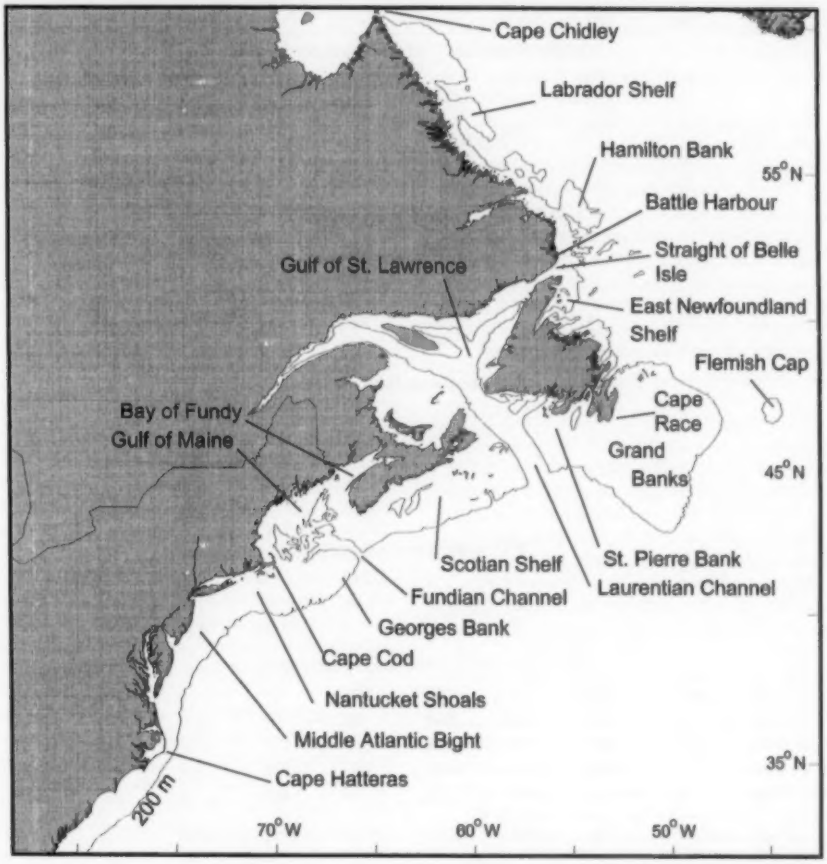
Fig. 1. Study area map with place names referred to in the text.



Hatteras, North Carolina, U.S.A., to Cape Chidley, Labrador, Canada, aggregated into bands about 30 nautical miles in width (Mahon and Sandeman 1985). To examine assemblages at a biogeographical scale, a data set that spans a large area is needed. In that regard, the scale of the present study is unique.

This paper describes the analysis of research demersal trawl survey data for the east coast of North America from Cape Hatteras in the south to Cape Chidley in the north (Fig. 1). The primary goal is to describe and map species assemblages and to evaluate evidence for ecological regime and assemblage distribution shifts on a decadal time scale. The questions addressed in this study are as follows. (*i*) What are the demersal fish assemblages? (*ii*) What are the assemblage distributions? (*iii*) Are the assemblages stable over time? (*iv*) How does the large-scale view of assemblage distribution relate to previous views of marine biogeography in the study area?

To answer these questions, three categories of analyses were undertaken: species by species descriptions of geographic distribution, multivariate analyses to define species assemblages, and preliminary evaluation of temporal variation in assemblage distributions. However, analyzing sample level data for such a large area poses a variety of problems. On one hand, constraints are imposed by the inability of most software available for assemblage analysis to accommodate such a large data set. On the other, advances in microcomputers and mapping software have made it practical to manipulate and visualize large data sets such as this one.

The data set

Data sources and selection

The data analyzed in this study were collected by demersal research trawl surveys from 1970 to 1994. The survey designs, stratification schemes, gears, etc., for these surveys have been described in several publications (Grosslein 1969; Doubleday 1981; Doubleday and Rivard 1981). Since these publications, there have been new vessels and gears used, and additional surveys have been carried out at other times of the year in various areas, but the basic designs have remained the same. The main trawl survey time-series in each area, which were established to provide indices of abundance for important commercial species, have been carried out continuously from the following years: 1963 in the United States, 1970 in the Scotia–Fundy and southern Gulf of St. Lawrence regions, 1983 in the northern Gulf of St. Lawrence, and various starting years prior to and during the 1970s off Newfoundland and Labrador.

In addition to information on abundance, these surveys also

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Table 1. Number of sets in the trawl survey data by source, 5-year period, and month.

Period	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
NMFS, Northeast Fisheries Science Center													
1970–1974			573	905	156				125	659	646		3 066
1975–1979			372	841	395		94	430	**222**	**960**	**628**	**54**	3 996
1980–1984	140	175	417	765	200	15	192	81	**270**	**767**	**272**		3 294
1985–1989		27	543	608					**303**	**747**	**109**	**1**	2 338
1990–1994		276	561	562			52	14	**358**	**531**			2 354
DFO, Scotia–Fundy region													
1970–1974						65	591	29					685
1975–1979			115				**624**	**86**		66	105	35	1 031
1980–1984		31	571	72			**720**	**13**	32	704	208		2 351
1985–1989		52	703	4		**11**	**844**	**17**		343			1 974
1990–1994		242	492			**53**	**935**	**2**					1 724
DFO, southern Gulf region													
1970–1974								1	314				315
1975–1979									**320**	**16**			336
1980–1984								**21**	**358**				379
1985–1989								**4**	**879**				883
1990–1994									**885**				885
DFO, northern Gulf region													
1980–1984	**395**						**112**	**8**	**9**				499
1985–1989	**790**						**46**	**850**	**13**				1 699
1990–1994	**635**							**760**	**424**				1 819
DFO, Newfoundland–Labrador region													
1970–1974	28	118	144	205	364	285	108	39	76	156	132	28	1 683
1975–1979	**296**	**410**	**39**	**339**	**816**	**544**	**66**	**358**	**375**	**406**	**541**	**52**	4 242
1980–1984	**396**	**593**	**268**	**457**	**795**	**391**	**103**	**174**	**236**	**416**	**1127**	**350**	5 306
1985–1989	**283**	**896**	**372**	**818**	**1319**	**479**	**143**	**710**	**164**	**641**	**1299**	**343**	7 467
1990–1994	**93**	**704**	**6**	**722**	**1117**	**345**	**39**	**485**	**6**	**384**	**2135**	**616**	6 652
Total	3056	3524	5176	6298	5162	2188	4669	4082	5369	6796	7202	1479	54 978

Note: NMFS, National Marine Fisheries Service (United States); DFO, Department of Fisheries and Oceans (Canada). The periods and months in bold type were used in the multivariate analysis.

collect data on size, sex, age, and reproductive condition. In view of the large size-related niche shifts exhibited by most fishes, assemblage analysis of size-structured data would be desirable (Werner and Gilliam 1984). However, including this level of detail would increase the volume of data by at least an order of magnitude and was considered to be impractical for the present study.

The abundance data from these surveys were combined into a single data set as described in Brown et al. (1996). The fish species captured by the trawl surveys were defined as demersal, pelagic, or mesopelagic based on information available in various texts (e.g., Bigelow and Schroeder 1953; Scott and Scott 1988). Only demersal species are included in this study. The only invertebrate considered to be sufficiently well sampled by the trawl gear is the shortfin squid, which was included in the analysis.

The data, which include only valid survey tows using standard survey gear, were acquired from five major sampling areas: (*i*) Labrador Shelf from Cape Chidley south to the Grand Banks of Newfoundland, Flemish Cap, and the west coast of Newfoundland, (*ii*) the northern Gulf of St. Lawrence, (*iii*) the southern Gulf of St. Lawrence, (*iv*) the Scotian Shelf, Bay of Fundy, and part of Georges Bank, and (*v*) Georges Bank, Gulf of Maine, and the Middle Atlantic Bight (Brown et al. 1996). The initial data set included all months in the years 1970–1994, comprising 54 978 trawl tows, with 26 286 369 individuals from 412 species, including some aggregate groups (Brown et al. 1996) (Table 1).

In several instances, specimens in the catch are not identified to species, and several aggregate groups were formed in the initial data processing by combining species for which there is a high probability of misidentification. However, the only aggregate group used in the final analyses is redfishes, which comprise three *Sebastes* species.

Data transformation

Because this data set consists of data from several sources, differences among the vessels and gears may have affected the catch per standard tow. Since there are some areas of overlap between surveys, and the same vessel and gear are sometimes used in more than one area, some limited intercalibration of vessels and gear would have been possible. However, previous studies have indicated that, even with comparative fishing experiments, intercalibration of vessels and gear is seldom statistically significant and that the coefficients are usually determined by only a few data points (Mahon and Smith

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1989*b*). Furthermore, since intercalibration data are not available for all vessels and gear types used in the different surveys over the years, it was not deemed feasible to convert all surveys to a single standard.

Number caught per tow was used as the abundance measure in the analyses, based on the assumption that gear and vessel biases are likely to be small relative to sampling variability and the major signals. Also, other approaches, such as analyzing presence–absence or data grouped into intervals, degrade the information but do not eliminate the potential biases. Because trawl survey data frequently have a skewed distribution, the data were $\log_{10}(x + 1)$ transformed. Studies on trawl survey data suggest that the differences in results obtained using biomass or numbers are likely to be negligible (Bianchi and Høisæter 1992).

Methods

The analytical objectives of this project are to identify species assemblages and to determine spatial and temporal patterns, but no single method is widely accepted for identifying and mapping species assemblages. Because each methodological approach has strengths and weaknesses, several methods were used in this study. The rationale is that if several distinct analyses show similar results, the overall robustness of the conclusions is enhanced. Available approaches range from qualitative analyses that depend on the familiarity of the investigator with distribution patterns to univariate approaches, such as plotting distribution maps, to multivariate analyses, such as classification (usually cluster analysis) and ordination (Clifford and Stephenson 1975; Gauch 1982).

The assemblage analysis began with a subjective visual classification of the single-species distribution patterns. A multivariate ordination procedure, principal components analysis (PCA), was then used to identify assemblages statistically. Although there are other ordination methods that were developed specifically for ecological data (e.g., Gauch 1982; Ter Braak 1986) and have been used successfully in other studies of demersal fishes (e.g., Bianchi 1992), the available software cannot process the large number of cases found in this trawl survey data set.

Finally, cluster analysis was conducted for comparison with the PCA. Cluster analysis was performed on (*i*) sites (i.e., tows) to generate mappable results comparable with maps of PCA scores and (*ii*) the species assemblages identified by PCA and cluster analysis to establish the hierarchical relationships among the assemblages.

Visual classification

Visual classification of species distributions was carried out as a background for interpreting the multivariate analyses. The purpose of this process was to develop an intuitive, knowledge-based classification that could be compared with the results of the more objective multivariate approaches. The distributions of 108 demersal species were mapped for all years and months combined using a $\log_{10}(x + 1)$ abundance scale. The maps were then subjectively classified into groups based on a subjective visual interpretation of the distributions, combined with the biological and other expert knowledge.

Multivariate analyses

The multivariate analyses were conducted using a subset of the trawl survey data which was developed as follows (Table 1). Preliminary PCA runs were tried with various subsets of months. Ultimately, the main survey series in each area was used in the multivariate analyses. The Newfoundland–Labrador surveys are carried out at different times of the year in different areas. Therefore, all months of Newfoundland–Labrador data were included in the data subset. The data used from the rest of the surveys were primarily from the summer and autumn. For the northern Gulf of St. Lawrence, data from July to September and January were used. For the southern Gulf of St. Lawrence, data from August to October were used. For the Scotian Shelf and Bay of Fundy, data from July to September were used. For Georges Bank south to Cape Hatteras, data from September to December were used.

Data were screened in 5-year time periods for consistency in spatial coverage. For 1970–1974, the Labrador Shelf was considered to be inadequately covered. Therefore, data prior to 1975 were excluded from the multivariate analyses.

Three species selection criteria were applied to ensure adequate sample size for each species in the multivariate analyses: (*i*) 500 individuals must have been caught for the entire 1975–1994 period, (*ii*) the species had to have been caught in at least 0.05% of the trawl sets for the 1975–1994 period, and (*iii*) 100 individuals had to have been caught in each 5-year period between 1975 and 1994.

The final data subset used for the multivariate analyses contained 1975–1994 data for 66 species collected in 38 695 tows (Tables 1 and 2).

PCA was used as the primary multivariate analysis method. Species loadings on the principal components (PCs) were used to identify groups of species that tend to co-occur (i.e., assemblages). Previous analyses have shown that ubiquitous species may be a member of more than one assemblage, and PCA allows a species to be associated with more than one component. The correlation matrix of $\log_{10}(x + 1)$-transformed number caught per tow was used as input for the PCA.

Two categories of species loadings on PCs are recognized in this study. Species with loadings >0.5 on a PC are considered the major species in the assemblage represented by that PC. Species with loadings between 0.3 and 0.5 are also considered members of assemblages, albeit with weaker associations. The usual practice, to use a loading of 0.5 (i.e., a PC contains 25% of the overall variability of a species) as the cutoff, is not based on statistical significance; it is merely a convention. The large size of the data set and the large number of PCs, which are distributed over a huge study area, enable recognition of the weaker class of associations (Gorsuch 1974).

The eigenvalue associated with a PC indicates the relative importance of that component. PCs with eigenvalues >1 are considered to represent statistically significant assemblages (Jolliffe 1986).

VARIMAX rotation was used for the PCs having eigenvalues >1 in the unrotated PC extraction. VARIMAX rotation is typically used when it is desirable to have a relatively small number of species strongly associated with (i.e., highly loaded on) each PC (Jolliffe 1986). VARIMAX rotation also tends to find a solution with few negative loadings on the PCs, thus facilitating interpretation of the PCs as assemblages.

The site scores on each VARIMAX-rotated PC are used as a measure of the extent to which the assemblages defined by the PCs were present at each site. Every assemblage can potentially be present at every site, as the assemblages are not mutually exclusive in space. Thus, groups of sites can be defined on the basis of PC scores. In this study, the "core" sites of assemblages were determined by selecting the sites in the top 5% of the site scores for each PC.

The large data set and software limitations precluded hierarchical agglomerative clustering for the entire data set, necessitating a two-stage analytical process in the cluster analyses of trawl tows (sites). This analysis was performed using the same subset of the groundfish data and transformation that was used for the PCA. The results of the site clustering are directly comparable with the mappable groups derived by selecting the sites with the top 5% of PC scores.

In the first stage, a random subset of 10% of the data (about 4000 tows) was clustered using squared Euclidean distance and Ward's method. To be consistent with the PCA, clustering for this data subset was stopped at 18 cluster groups (CGs). The second stage was a nonhierarchical clustering method, nearest centroid sorting, that requires cluster "seed points" as initial estimates of the cluster means (Anderberg 1973; SAS Institute Inc. 1989). Output from the first

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Table 2. Numbers of individuals caught per 5-year time period for species with a total catch >500 and catch >100 in each period.

Common name	Scientific name	Time period			
		1975–1979	1980–1984	1985–1989	1990–1994
Redfishes	*Sebastes* spp.	2 291 514	2 581 080	2 259 409	2 007 064
American plaice	*Hippoglossoides platessoides*	585 609	632 769	864 637	480 839
Atlantic cod	*Gadus morhua*	221 160	399 575	961 117	589 339
Butterfish	*Peprilus triacanthus*	134 043	241 423	211 793	159 600
Silver hake	*Merluccius bilinearis*	101 697	104 676	187 476	111 989
Greenland halibut	*Reinhardtius hippoglossoides*	91 544	91 306	151 697	124 259
Arctic cod	*Boreogadus saida*	249 455	40 196	86 902	47 883
Yellowtail flounder	*Limanda ferruginea*	95 950	78 101	118 593	121 554
Haddock	*Melanogrammus aeglefinus*	68 258	117 574	120 027	61 085
Shortfin squid	*Illex illecebrosus*	125 184	75 618	59 490	89 368
Northern sand lance	*Ammodytes dubius*	70 832	153 408	10 590	13 390
Spiny dogfish	*Squalus acanthias*	47 280	52 799	70 042	64 776
Thorny skate	*Raja radiata*	35 759	46 618	74 342	71 763
Witch flounder	*Glyptocephalus cynoglossus*	38 102	43 771	66 544	52 929
Black dogfish	*Centroscyllium fabricii*	6 395	14 530	52 416	68 160
White hake	*Urophycis tenuis*	23 378	23 381	55 643	27 173
Winter flounder	*Pseudopleuronectes americanus*	16 418	16 878	48 991	46 252
Atlantic argentine	*Argentina silus*	20 591	13 507	57 278	19 325
Longhorn sculpin	*Myoxocephalus octodecemspinosus*	21 224	14 053	32 001	34 306
Rock (roundnose) grenadier	*Coryphaenoides rupestris*	34 515	22 708	31 838	5 402
Marlin-spike (common grenadier)	*Nezumia bairdi*	13 618	12 181	16 969	32 601
Red hake	*Urophycis chuss*	22 035	18 132	19 873	11 414
Lumpfish	*Cyclopterus lumpus*	3 063	4 936	26 079	25 777
Scup	*Stenotomus chrysops*	28 115	10 641	16 776	3 580
Spotted hake	*Urophycis regius*	18 782	14 778	16 039	7 058
Roughhead grenadier	*Macrourus berglax*	14 757	15 086	12 184	12 803
Pollock	*Pollachius virens*	4 589	7 908	15 606	13 310
Atlantic wolffish	*Anarhichas lupus*	13 179	10 479	8 519	5 533
Longfin hake	*Urophycis chesteri*	1 864	7 013	12 018	11 538
Little skate	*Raja erinacea*	10 047	7 875	7 591	5 839
Fourspot flounder	*Paralichthys oblongus*	6 890	8 908	6 960	4 574
Moustache (mailed) sculpin	*Triglops murrayi*	5 760	5 852	7 855	7 382
Winter skate	*Raja ocellata*	3 949	6 808	7 266	4 725
Windowpane	*Scophthalmus aquosus*	6 885	3 701	7 022	2 767
Gulf Stream flounder	*Citharichthys arctifrons*	3 706	5 301	3 979	3 431
Sea raven	*Hemitripterus americanus*	3 241	3 568	5 099	3 700
Northern (common) searobin	*Priondius carolinus*	4 924	2 486	3 470	3 721
Northern wolffish	*Anarhichas denticulatus*	5 305	4 953	2 318	862
Smooth skate	*Raja senta*	3 351	3 198	3 734	2 919
Roughnose grenadier	*Trachyrhynchus murrayi*	1 405	957	6 500	137
Blue hake	*Antimora rostrata*	2 785	2 647	1 396	1 695
Spotted wolffish	*Anarhichas minor*	2 182	2 787	1 674	962
Goosefish (angler)	*Lophius americanus*	1 889	1 734	1 816	1 464
Blackbelly rosefish	*Helicolenus dactylopterus*	2 125	1 474	1 140	1 562
Ocean pout	*Macrozoarces americanus*	1 698	1 403	1 622	1 534
Fourbeard rockling	*Enchelyopus cimbrius*	716	607	3 041	1 356
Atlantic sea poacher	*Agonus decagonus*	1 495	307	1 359	2 302
Alligatorfish	*Aspidophoroides monopterygius*	695	480	2 527	1 645
Fawn cusk-eel	*Lepophidium cervinum*	1 176	1 518	1 622	966
Atlantic halibut	*Hippoglossus hippoglossus*	1 186	1 204	1 229	785
Shorthorn sculpin	*Myoxocephalus scorpius*	273	454	1 717	1 767
Longnose eel	*Synaphobranchus kaupi*	860	530	668	1 328
Black sea bass	*Centropristis striata*	2 061	421	418	354
Cunner	*Tautogolabrus adspersus*	146	224	2 196	605
Atlantic hagfish	*Myxine glutinosa*	963	573	389	1 157
Polar sculpin	*Cottunculus micropes*	434	295	994	1 240
Snowflake hookear sculpin	*Artediellus uncinatus*	253	158	1 337	999

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Table 2 (*concluded*).

Common name	Scientific name	Time period			
		1975–1979	1980–1984	1985–1989	1990–1994
Large scale tapirfish	*Notocanthus chemnitzi*	887	862	658	291
Offshore hake	*Merluccius albidus*	660	1 103	353	123
Cusk	*Brosme brosme*	794	502	474	236
Spinytail skate	*Raja spinicauda*	394	490	365	282
Snake blenny	*Lumpenus lumpretaeformes*	187	252	304	677
Shortnose greeneye	*Chlorophthalmus agassizi*	113	361	731	166
Greenland cod	*Gadus ogac*	401	147	305	490
Summer flounder	*Paralichthys dentatus*	624	313	160	104
Smooth dogfish	*Mustelus canis*	610	120	147	110

stage, mean values for each species in each of the subset CGs, was used as seed points for the second stage, which was performed using the entire data set, and produced 18 site CGs consisting of various numbers of individual trawl tows.

Numerous similarity–dissimilarity coefficients are available (Clifford and Stephenson 1975), and the Bray–Curtis coefficient has been shown to best reflect relationships among species and sites in ecological studies of species distribution data (Bloom 1981). However, the Bray–Curtis coefficient could not be used in this study because it is not available in any software package capable of clustering a sufficiently large number of records.

Species associations with the site CGs were determined by calculating a standardized abundance for every species in each site cluster. The calculation was performed by dividing the mean $\log_{10}(x + 1)$ catch per tow for each site CG by the mean $\log_{10}(x + 1)$ catch per tow for the entire database. This value is considered an index of the affinity of a species for the CGs, with high values indicating strong affinity. Species were assigned to the site CG for which they had their highest standardized abundance. Therefore, the methods used for the sites cluster analysis limited the species to only one assemblage.

Neither PCA nor the nonhierarchical clustering method used in the second stage of the cluster analysis provides a measure of the hierarchical interrelationships among assemblages (PCs or CGs). These are important for identifying the relative strength of biogeographical discontinuities that could be the basis for defining ecosystems or spatial units for management. Therefore, the PCs and CGs were clustered using squared Euclidean distance and Ward's method to determine these hierarchical relationships. The PCs were clustering using the species loadings on the PCs as the input data. The CGs were clustered using the mean $\log_{10}(x + 1)$ catch per tow for each species in each CG as the input data.

Temporal analyses

Two aspects of temporal variation in the PCA assemblages were examined: (*i*) temporal stability of assemblage structure and (*ii*) temporal variability of spatial distribution. Temporal stability of assemblage structure was examined by running separate PCAs on data for each of four 5-year periods between 1975 and 1994. Species loadings on the PCs were then compared among the periods and with the loadings for the PCA of the entire 1975–1994 period. However, objective statistical methods for these comparisons are not available.

Temporal variation in the spatial distribution of the 1975–1994 assemblages was investigated for PC10 (thorny skate, American plaice, witch flounder, Atlantic cod, smooth skate) and PC11 (Arctic cod, Atlantic sea poacher, Greenland halibut, polar sculpin). The sites within the top 5% of scores for these components were mapped by 5-year time periods. Mean latitudes and longitudes for the 5-year time periods were compared using analysis of variance. Prevalence of each assemblage over time was assessed by totaling the number of sites in the top 5% of scores for PCs 10 and 11 (as determined from analysis of the entire data set) in each 5-year time period. The assumption in this approach is that particular assemblages exist throughout the study period, but their spatial distributions may vary.

An assumption in the temporal analyses is that entire species distributions are well sampled throughout the 1975–1994 period and that there are no sampling biases through space and time. This assumption is not completely met (e.g., sampling was discontinued on the Flemish Cap in the mid-1980s), so sampling biases may have had some impact on temporal patterns detected. This problem is likely to be more important for species at the periphery of the study area, where sampling intensity may have been variable, than for species widely distributed throughout the core of the study area.

Results

Visual classification

The species plots were classified into nine groups (Table 3; Fig. 2). Three groups occurred principally in water >200 m deep. The Northern Deepwater group is generally found north of the Scotian Shelf, the Temperate Deepwater group typically extends from the Gulf of St. Lawrence to the Gulf of Maine, and the Southern Deepwater group typically occurs south of the Scotian Shelf.

Four bank and slope groups, which occur principally in water <200 m deep, were identified. The Northern and North-temperate Bank/Slope groups range from the eastern Scotian Shelf to Cape Chidley and from Georges Bank to Cape Chidley, respectively. The South-temperate Bank/Slope group is the largest, with typical ranges extending from the Middle Atlantic Bight to the Grand Banks of Newfoundland. The Southern Bank/Slope group extends from the eastern Scotian Shelf or southern Gulf of St. Lawrence to Cape Hatteras. Because the data set is sparse south of Cape Hatteras, the Southern Transitional group, which contains species primarily occurring south of Cape Hatteras, is not portrayed.

Principal components analysis

The results of the PCAs for the entire 1975–1994 time period and for the 5-year periods were similar. The analysis of the 1975–1994 period yielded 19 PCs with eigenvalues >1, which cumulatively explained 56.3% of the variance. The PCAs of the 5-year periods each yielded 19 PCs with eigenvalues >1, cumulatively explaining 58.0–59.4% of the total variance within the 5-year data sets. These results suggest that the entire time-series can be analyzed in one PCA.

The species with loadings >0.3 on the VARIMAX-rotated PCs with eigenvalues >1 are shown for the entire time period in Table 4. PCs were associated with one to five species with

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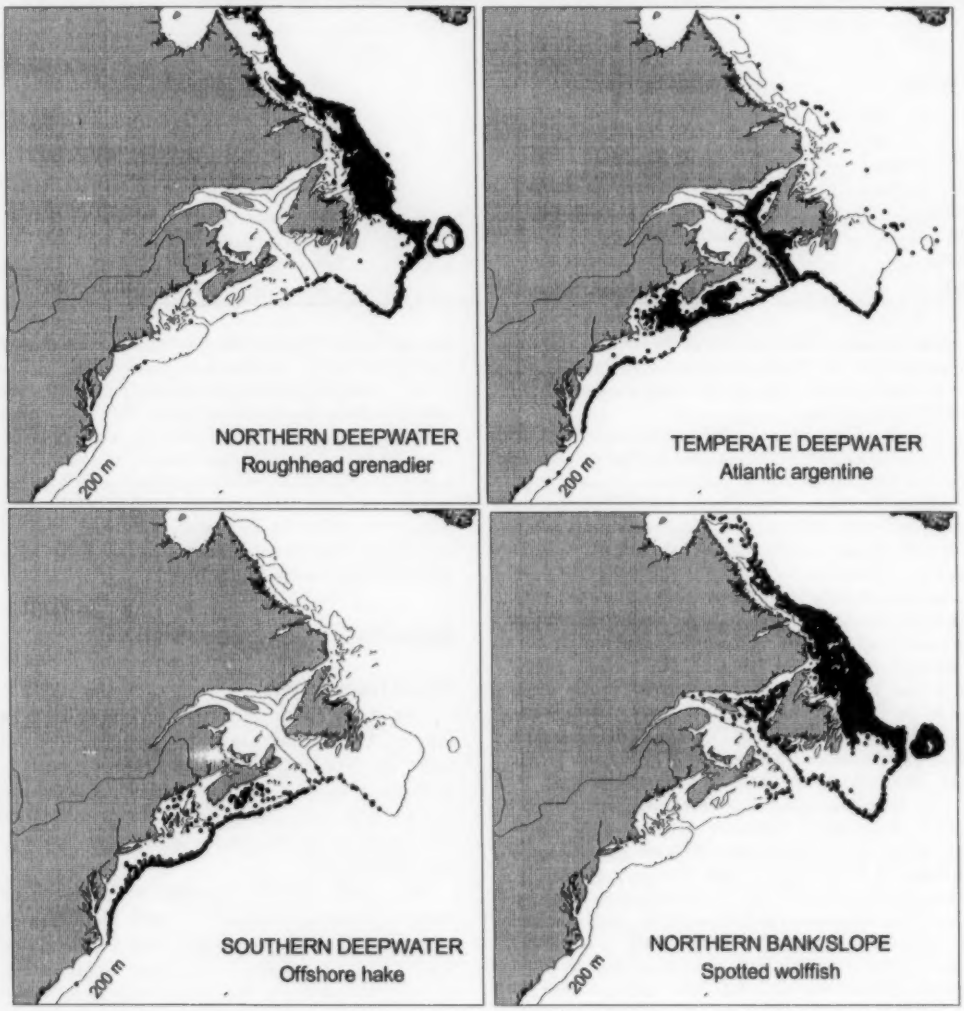
Fig. 2. Maps of example species for the species groups determined by visual classification of species distribution maps.



loadings >0.5 and with zero to five species with loadings between 0.5 and 0.3. Under the loadings >0.5 criterion for high-level inclusion in an assemblage, 54 of the 66 species were members of an assemblage, but only one species, black dogfish, was a member of two. Under the loadings >0.3 criterion for low-level inclusion in an assemblage, two species, roughhead grenadier and Greenland halibut, were members of three assemblages, 15 species were members of two assemblages, and two species, spinytail skate and Greenland cod, were not members of any assemblage. Species with very high loadings on one PC, such as blue hake, roughnose grenadier, and Gulf Stream flounder, may be considered assemblage specialists (stenotopic); species with low loadings on more than one PC may be considered assemblage generalists (eurytopic). Species with loadings <0.3 are not considered members of any assemblage.

Mapping the high 5% of scores on each PC, assumed to represent the distribution core of the assemblage associated with that PC, indicated a high degree of spatial aggregation of the sites and a wide variety of distribution patterns (Fig. 3). The decision to use the high 5% of scores on each PC was based on mapping and examining the high 5, 10, 15, and 20% on several PCs (Brown et al. 1996). As the percentage included in the map was increased, the spatial aggregation of the assemblages decreased and the assemblages became progressively less spatially distinct.

Some assemblages are clearly associated with deep-slope areas (e.g., PCs 2, 6, 8, 10, 16), while others occur primarily on the tops of banks (e.g., PCs 1, 3, 4, 5, 7, 13, 17, 18). The distribution patterns of the assemblages show that they occur at spatial scales that can be detected with trawl surveys and that can be perceived at the overall scale of the study. The degree to which these assemblages are biologically functional units, or merely consist of species with similar responses to environmental gradients, remains unknown. For some assemblages (e.g., PC5), it appears that the topographic and

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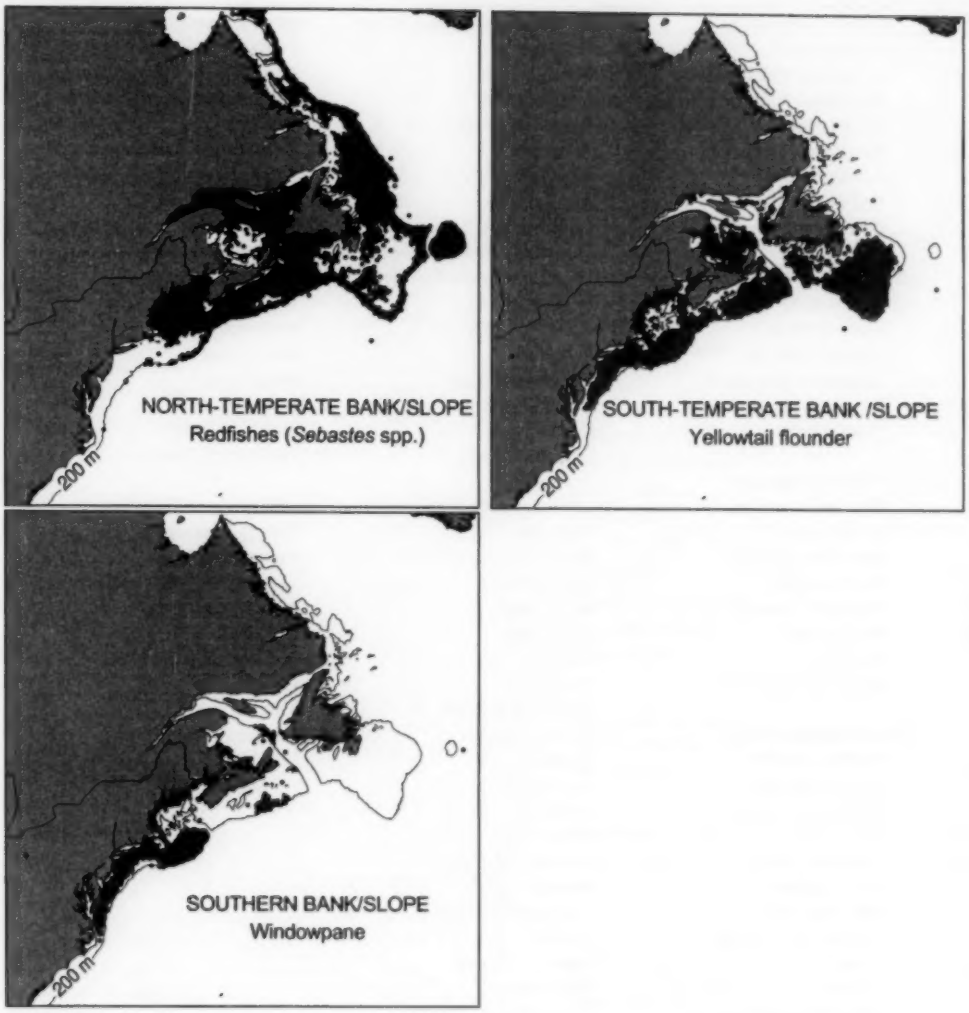
Fig. 2 (*concluded*).



hydrographic complexity of the shelves provides patches of appropriate habitat that are scattered throughout the study area.

In contrast with the site cluster analysis, where each site is assigned to a particular assemblage group, the PCA approach allows more than one assemblage to occur at a site and also allows no assemblage to occur at a site (Table 5). Just under half of the sites (45.5%) did not fall into the top 5% of scores on any PC. Assuming that the 5% selection criterion is a good measure of assemblage integrity, this indicates that assemblages can only be identified at 54.5% of the sites. The fish fauna at the remaining 45.5% of sites would, therefore, be considered unstructured. Only one assemblage was detected at 31.8% of the sites, while more than one assemblage occurred at 22.7% of sites, with 10 sites having as many as eight assemblages.

One problem with using site scores from PCA is that an assemblage may be shown at a site where one of the major species of that assemblage does not occur. PC15, Atlantic halibut/haddock, provides a simple example (Fig. 3). No haddock were caught north of Hamilton Bank, yet the assemblage is shown as present along the slope of the Labrador Shelf because Atlantic halibut is relatively abundant there. Similarly, although the map of PC17 (Fig. 3) shows the area where lumpfish and shorthorn sculpin co-occur around St. Pierre Bank, it also shows this assemblage on the shelf northwest of Newfoundland, where the latter species was seldom caught.

An additional problem with the correlation matrix based PCA method used is that associations involving ubiquitous species are deemphasized because assemblages are defined by species that tend to co-occur at unusually high frequencies, even if they are not particularly abundant overall. For example, gulfstream and fourspot flounder are the species with the highest loadings on PC1 (0.81 and 0.76, respectively), which explains 8.8% of the total variance (Table 4). Although they are not particularly abundant, these species both occur primarily south of Cape Cod. In contrast, widespread or abundant

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Table 3. Species groups determined by visual classification of distribution plots for the entire data set.

Northern Deepwater	North-temperate Bank/Slope	Southern Bank/Slope
Longnose eel	Redfishes	Fourspot flounder
Largescale tapirfish	Witch flounder	Butterfish
Blue hake	Smooth skate	Spotted hake
Rock (roundnose) grenadier	Atlantic wolffish	Fawn cusk-eel
Roughhead grenadier	Atlantic cod	Gulf Stream flounder
Spinytail skate	American plaice	Summer flounder
Stoutsaw palate	Thorny skate	Scup
Black herring	Alligator fish	Black sea bass
	Snowflake hookear sculpin	Northern (common) searobin
Temperate Deepwater	Moustache (mailed) sculpin	Smooth dogfish
Marlin-spike	Daubed shanny	Windowpane
Black dogfish	Atlantic halibut	Little skate
Atlantic argentine	Lumpfish	Bigeye scad
Roughnose grenadier	Shorthorn sculpin	Rough scad
Longfin hake	Snake blenny	Round scad
Barracudinas	Cunner	Plainhead filefish
	Radiated shanny	Smallmouth flounder
Southern Deepwater		
Blackbelly rosefish	**South-temperate Bank/Slope**	**Southern Transitional**
Offshore hake	Red hake	Vermilion snapper
Shortnose greeneye	Goosefish (angler)	Snake fish
Shortfin squid	Spiny dogfish	Tomtate
Armoured searobin	Silver hake	Inshore lizard fish
Buckler dory	White hake	Bank cusk-eel
Beardfish	Pollock	Offshore lizard fish
Slatjaw cutthroat eel	Cusk	Tattler
	Yellowtail flounder	Bank sea bass
Northern Bank/Slope	Winter flounder	Atlantic croaker
Northern wolffish	Ocean pout	Spot
Spotted wolffish	Sea raven	Longspine porgy
Atlantic sea poacher	Longhorn sculpin	
Arctic cod	Winter skate	
Greenland halibut	Northern sand lance	
Polar scuplin	Atlantic hagfish	
Greenland cod	Fourbeard rockling	
Fourline snake blenny	Haddock	
Threebeard rockling	Atlantic soft pout	
Atlantic spiny lumpsucker	Wrymouth	
Atlantic hookear sculpin	Threespine stickleback	

species, which may co-occur with many other species over their ranges, are much less strongly associated with assemblages identified by the PCA. For example, American plaice and Atlantic cod are caught in large numbers throughout the study area. American plaice is fairly strongly associated with PC10, and Atlantic cod is weakly associated with PCs 8 and 10. These two components explain only 2.6 and 2.4% of the total variance, respectively.

The pattern described above can also be seen in Table 6, which summarizes species compositions of PCs 10 and 11, using the top 5% of sites. Mean absolute catch per tow shows that the core species may actually be less abundant than other species (e.g., Atlantic sea poacher and American plaice for PC11). Relative catch per tow, calculated by dividing the mean catch per tow for the sites with the top 5% of PC scores by the overall mean catch per tow, provides a better, although not perfect, indication of the basis for the PCA extraction of assemblages.

The above problem occurred because the PCA approach has the implicit assumption that assemblages are determined by species that tend to occur primarily together, and not by widespread species that co-occur with many other species. However, widespread species may be present in considerable numbers where these assemblages occur, and they may play an important role in assemblage dynamics. To gain a better understanding of how PCA site scores relate to species composition, it would be useful to examine changes in species composition of subsets of sites with different scores on several PCs (e.g., top 5, 5–10, 10–15%, etc.). An alternative approach to defining the assemblages would be to use abundance of the major species identified by the PCA at some specified level of abundance or relative abundance (e.g., greater than the overall average catch per tow) as a cutoff, rather than the site scores. In this case, an assemblage could be considered present at sites where all the major species occurred in significant numbers.

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Table 4. Species loadings on VARIMAX-rotated PCs for the 1975–1994 data subset.

Species	Loading	Species	Loading
PC1		PC8	
Gulf stream flounder	0.808	**Spotted wolffish**	0.729
Fourspot flounder	0.762	**Atlantic wolffish**	0.726
Fawn cusk-eel	0.744	**Northern wolffish**	0.676
Spotted hake	0.663	Roughhead grenadier	0.428
Butterfish	0.528	Atlantic cod	0.366
Red hake	0.388	Greenland halibut	0.345
Goosefish (angler)	0.330	Redfishes	0.303
PC2		PC9	
Blue hake	0.843	**Blackbelly rosefish**	0.792
Rock grenadier	0.770	**Offshore hake**	0.775
Longnose eel	0.763	**Shortnose greeneye**	0.680
Large-scale tapirfish	0.663		
Roughhead grenadier	0.506	PC10	
Marlin-spike (common grenadier)	0.301	**Thorny skate**	0.687
		American plaice	0.637
PC3		Witch flounder	0.415
Scup	0.796	Atlantic cod	0.372
Summer flounder	0.752	Smooth skate	0.356
Northern (common) searobin	0.751		
Black sea bass	0.725	PC11	
Smooth dogfish	0.578	**Arctic cod**	0.751
		Atlantic sea poacher	0.742
PC4		Greenland halibut	0.451
Silver hake	0.689	Polar sculpin	0.304
Red hake	0.574		
Cusk	0.550	PC12	
Pollock	0.539	**Fourbeard rockling**	0.681
Spiny dogfish	0.512	**Atlantic hagfish**	0.619
White hake	0.467	Smooth skate	0.316
Ocean pout	0.405		
Goosefish (angler)	0.380	PC13	
Haddock	0.361	**Moustache (mailed) sculpin**	0.693
Shortfin squid	0.350	**Alligatorfish**	0.647
		Snowflake hookear sculpin	0.604
PC5			
Longhorn sculpin	0.771	PC14	
Sea raven	0.673	**Roughnose grenadier**	0.831
Yellowtail flounder	0.634	**Longfin hake**	0.604
Winter flounder	0.598	**Black dogfish**	0.513
Ocean pout	0.468		
		PC15	
PC6		**Atlantic halibut**	0.790
Marlin-spike (common grenadier)	0.727	**Haddock**	0.587
Redfishes	0.635		
Witch flounder	0.529	PC16	
Black dogfish	0.525	**Polar sculpin**	0.530
Atlantic argentine	0.492	Roughhead grenadier	0.365
White hake	0.443	Greenland halibut	0.357
Longfin hake	0.352		
		PC17	
PC7		**Lumpfish**	0.778
Windowpane	0.755	**Shorthorn sculpin**	0.635
Winter skate	0.748	Sea raven	0.339
Little skate	0.722		
Northern sand lance	0.387	PC18	
		Cunner	0.630
		Winter flounder	0.390
		Snake blenny	0.361

Note: Bold type indicates species with loadings >0.5; standard type indicates species with loadings >0.3 and <0.5.

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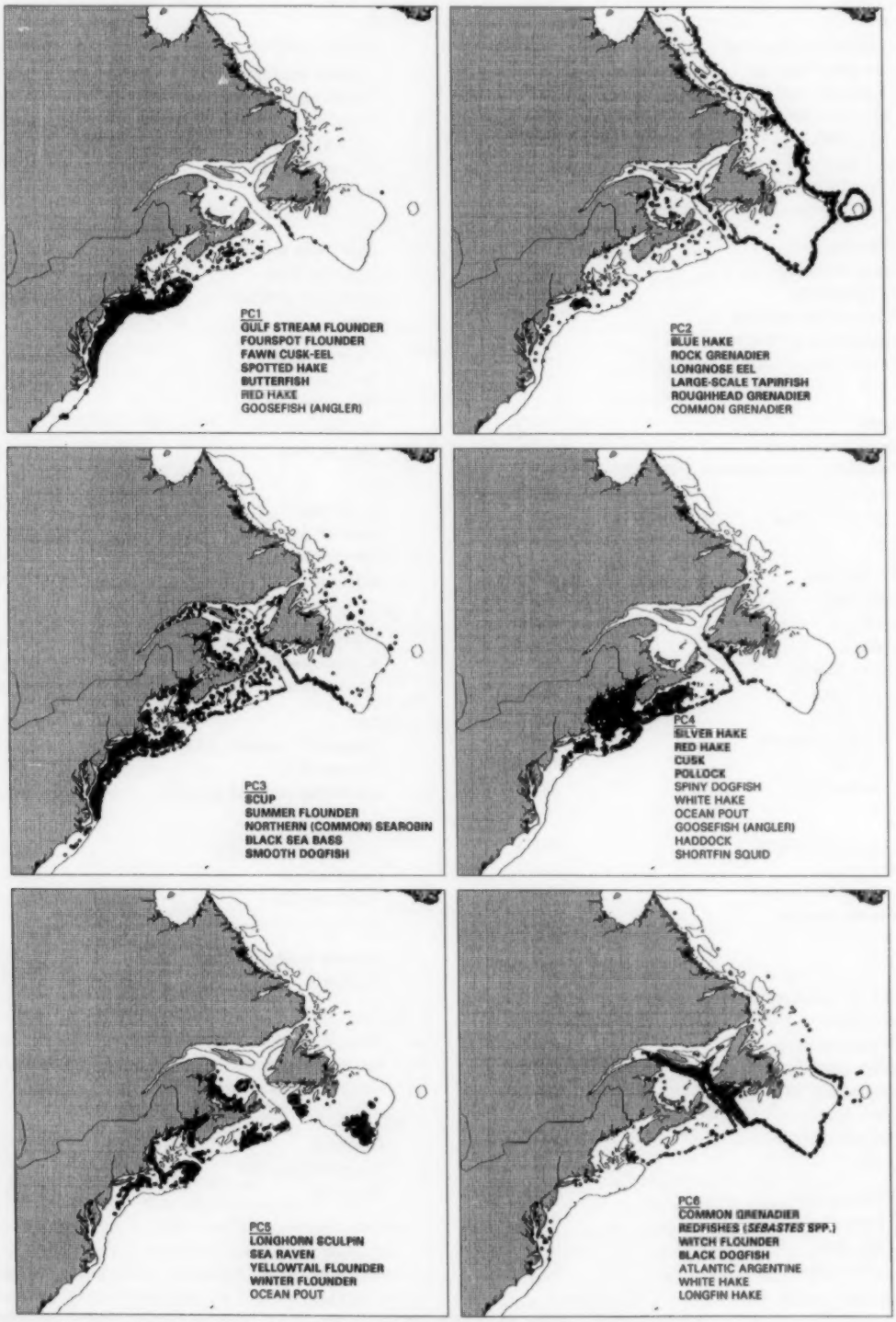
Fig. 3. Maps of groundfish assemblages, based on the top 5% of scores for PCs 1–18 derived from the 1975–1994 data. The species compositions shown are taken from Table 4.



Note: Bold type indicates species with loadings >0.5; standard type indicates species with loadings >0.3 and <0.5.

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Fig. 3 (*continued*).

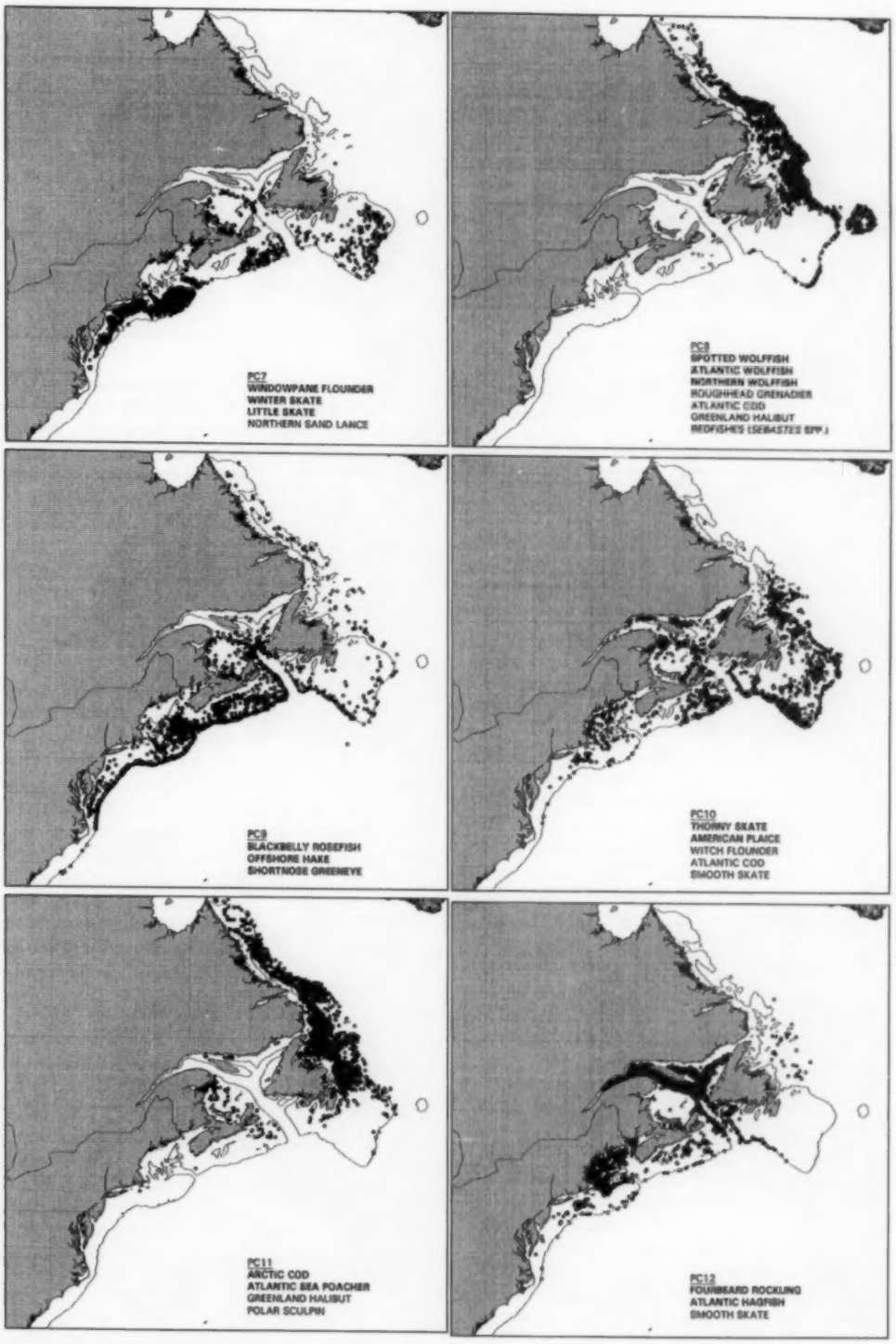

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Fig. 3 (*concluded*).

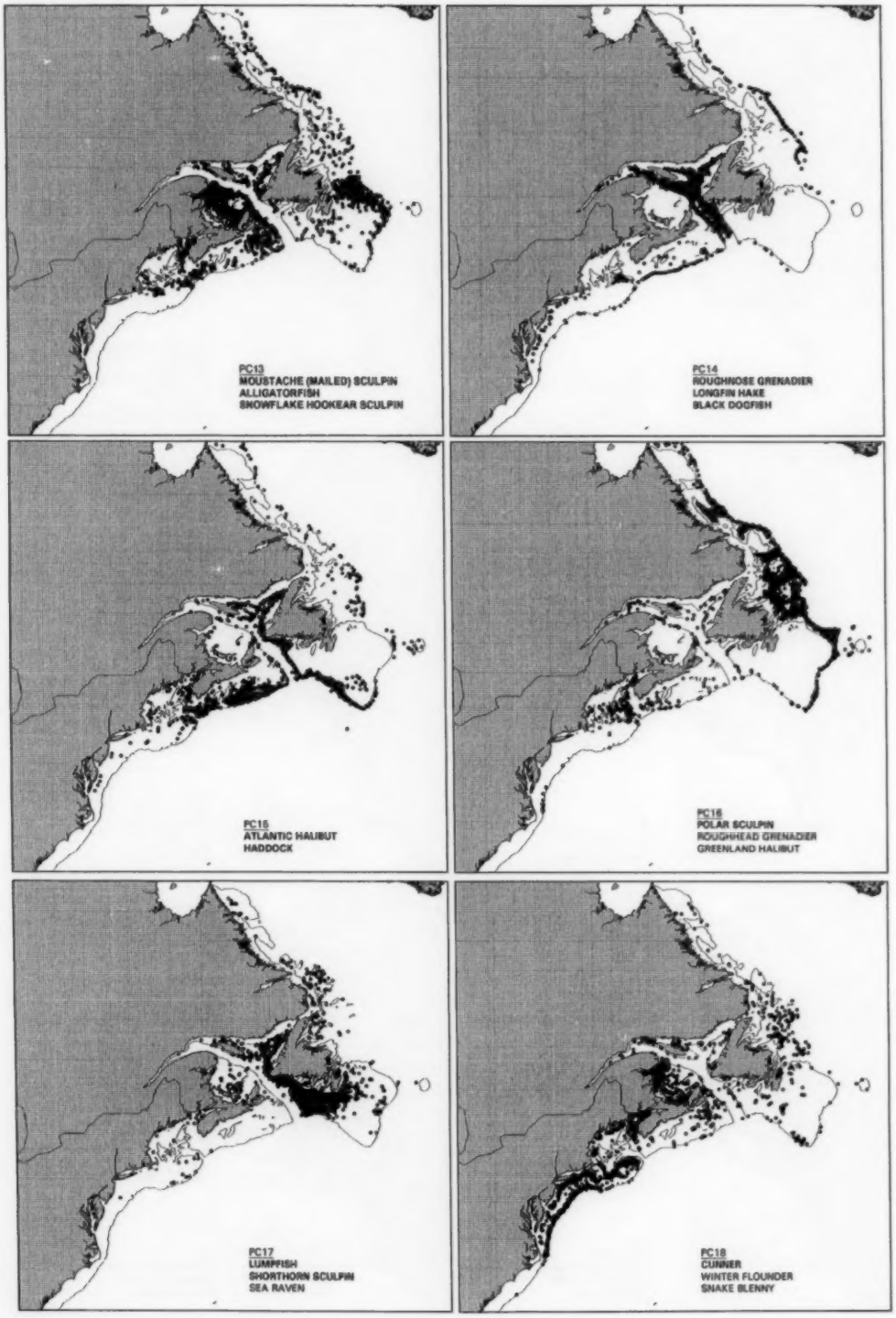

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Table 5. Number of sites without an assemblage, or with more than one assemblage, based on the criterion of selecting sites with scores in the top 5% on each PC.

No. of assemblages	No. of sites	% of sites
0	17 612	45.5
1	12 292	31.8
2	5 624	14.5
3	2 023	5.2
4	762	1.9
5	266	0.7
6	98	0.3
7	43	0.1
8	10	<0.1

Cluster analysis

Species compositions of the 18 site CGs are shown in Table 7. The numeric value in the table is the relative abundance of a species in a site CG (the average $\log_{10}(x+1)$-transformed number caught per tow of the species in that site CG divided by its average $\log_{10}(x+1)$-transformed number caught per tow in the entire data set, including the trawl sets that did not contain the species). Each species is assigned to the CG in which it has its highest relative abundance.

Species compositions of the 18 CGs ranged from zero to eight species (Table 7). Two CGs, 1 and 7, did not contain any species with their highest relative abundance. Unlike PCA, the methods used to identify species compositions of the CGs constrain each species to be associated with only one CG. However, maximum relative abundances vary considerably. Three species, thorny skate, American plaice, and Atlantic cod, had maximum relative abundances <2. These species are loosely associated with several CGs and occur at lower relative abundances in most of the remaining groups. They may be considered assemblage generalists (eurytopic). Three other species, snowflake hookear sculpin, roughnose grenadier, and black sea bass, had maximum relative abundances >60, indicating that they were strongly associated with their primary CG. These species tended to associate with only a few assemblages, were absent from many of the assemblages, and may be considered assemblage specialists (stenotopic).

The majority of the CGs show a considerable degree of spatial aggregation (Fig. 4). However, CG1, which contains 16 475 sites (42.6% of the sites) and did not contain any species with its highest relative abundance, is distributed over the entire study area. CG7 also has no associated species, but is distributed only from the Gulf of Maine south. These two CGs can be interpreted as groups of sites without any distinguishing assemblages. Accordingly, the remaining 16 CGs can be interpreted as assemblages. The sites in CG1 probably have several widespread species occurring in average or below-average abundance. CG1 contains 42.6% of the sites, which is consistent with the number of sites that did not fall into any PC assemblage (45.5%) under the top 5% of site scores criterion.

Depth and temperature characterization of PCs and CGs

The site scores on the PCs were used to characterize the depths and temperatures at which the assemblages occur. The average depths and temperatures were calculated for the sites in the top 5% of scores for each PC (Table 8). Mean bottom temperatures range from 1.0 to 11.2°C; mean depths range from 52 to 537 m. Substantial variation is evident, as standard deviations are 70 and 60% of the mean bottom temperatures and depths, respectively. However, assemblages close to each other in mean bottom temperature tend to be further apart in mean depth, and vice versa. For example, PCs 11 and 17 are consecutive on the temperature list (Table 8), with mean temperatures of 1.0 and 1.2°C. However, in the depth list, there are five PCs between them, and they have mean depths of 131 and 261 m.

Average depth and bottom temperatures were also calculated for the 18 CGs (Table 8). The patterns are similar to those observed for the PCA. Mean bottom temperatures range between 0.7 and 11.9°C; mean depths range between 58 and 504 m. Standard deviations are 79 and 64% of the mean bottom temperatures and depths, respectively.

The temperature and depth preferences of the assemblages shown in Table 8 may have some biases within them and should not be overinterpreted. The data used for the multivariate analyses included data from different months and seasons, which could distort the apparent temperature and depth preferences of the assemblages. This is most likely to have occurred for shallow assemblages. At depths over 200 m, seasonal variability is minimal. Developing a more refined analysis of assemblage temperature and depth preferences awaits further work.

Temporal variation

The assemblages identified by PCA of the entire 1975–1994 time period are compared with those identified by PCA of the 5-year time periods (Table 9). Species loadings on the PCs for the individual 5-year time periods are in Brown et al. (1996, appendix 4). There was considerable persistence of the 1975–1994 assemblages through time. Six assemblages (PCs 1, 7, 9, 10, 11, 12) contained a consistent core of high-level species (i.e., with loadings >0.5) throughout, although other species sometimes joined these assemblages during certain 5-year periods. Five assemblages (PCs 2, 5, 8, 17, 18) always retained their high-level species, but some of the loadings dropped below 0.5 during one or more 5-year periods. Five assemblages (PCs 3, 4, 6, 13, 14) split into two assemblages during at least one 5-year period. Three assemblages (PCs 6, 14, 15) disappeared from one 5-year period, but generally retained their high-level species during the other periods.

The assemblages for the entire time period were judged to be sufficiently consistent with those for 5-year time periods that the former could be used to represent the overall assemblages (Table 9). This result suggests that assemblages are fairly stable entities, but that their development and distribution may vary through time as species distributions and abundances fluctuate.

Examining temporal shifts in location for assemblages PC10 and PC11 (Fig. 5; Table 10) revealed substantial distribution shifts over the four 5-year time periods. However, the extent to which these were shifts of entire assemblages, or were only shifts in one of the major species, has not been determined.

Sites scoring in the top 5% of the scores for PC10 (thorny

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Table 6. Species loadings, mean catch per tow, and relative catch per tow of the top 5% of sites for PCs 10 and 11 (1975–1994 data).

PC10				PC11			
		Catch per tow				Catch per tow	
Loading	Species	Mean	Relative	Loading	Species	Mean	Relative
0.687	Thorny skate	40.06	6.83	0.751	Arctic cod	298.15	21.24
0.637	American plaice	183.68	2.78	0.742	Atlantic sea poacher	2.51	18.71
0.415	Witch flounder	31.70	6.14	0.451	Greenland halibut	72.53	6.07
0.372	Atlantic cod	129.07	2.41	0.304	Polar sculpin	0.54	7.45
0.356	Smooth skate	2.54	7.27	0.258	Greenland cod	0.55	16.03
0.189	Goosefish (angler)	0.97	5.49	0.162	Northern wolffish	2.07	3.69
0.182	Yellowtail flounder	27.00	2.46	0.123	Alligatorfish	0.40	2.88
0.178	Snake blenny	0.48	12.99	0.079	Shorthorn sculpin	0.17	2.81
0.142	Greenland halibut	25.89	2.19	0.066	Spotted wolffish	0.64	2.60
0.128	Spinytail skate	0.17	4.00	0.063	American plaice	96.74	1.40
0.098	Moustache (mailed) sculpin	1.19	1.72	0.062	Snake blenny	0.10	2.45
0.089	Red hake	8.26	4.37	0.034	Roughhead grenadier	1.26	0.81
0.067	Spotted wolffish	0.29	1.77	0.031	Atlantic halibut	0.05	0.44
0.053	Northern sand lance	12.71	1.11	0.026	Ocean pout	0.06	0.33
0.051	Atlantic wolffish	0.88	0.92	0.019	Red hake	0.01	0.00
0.048	Fourbeard rockling	0.39	2.88	0.019	Black sea bass	0.05	0.17
0.046	Atlantic sea poacher	0.18	1.21	0.012	Northern searobin	0.49	0.71
0.042	Roughnose grenadier	0.28	0.99	0.012	Gulf Stream flounder	0.00	0.00
0.038	Lumpfish	3.90	2.10	0.009	Little skate	0.05	0.04
0.032	Ocean pout	0.70	3.76	0.008	Spinytail skate	0.06	1.23
0.031	Roughhead grenadier	1.69	1.17	0.006	Goosefish (angler)	0.00	0.00
0.029	Polar sculpin	0.12	1.96	0.006	Fourspot flounder	0.04	0.04
0.022	Shortnose greeneye	0.30	6.48	0.005	Winter flounder	2.07	0.76
0.018	Atlantic halibut	0.24	2.30	0.002	Roughnose grenadier	0.00	0.00
0.015	Silver hake	24.67	1.93	0.001	Windowpane	0.07	0.11
0.015	Gulf Stream flounder	2.00	4.40				
0.010	Snowflake hookear sculpin	0.15	2.15				
0.009	Greenland cod	0.03	0.91				
0.007	Sea raven	0.50	1.22				
0.003	White hake	5.12	1.71				

skate, American plaice, witch flounder, Atlantic cod, smooth skate) and PC11 (Arctic cod, Atlantic sea poacher, Greenland halibut, polar sculpin) are mapped in 5-year intervals (Fig. 5). Because of the stability of assemblages, this was deemed an effective way to examine temporal variability in assemblage distribution. However, the cessation of sampling on the Flemish Cap in the mid-1980s may have introduced an apparent westward movement in distribution for the 1990–1994 period.

Prevalence of the PC10 assemblage, a north-central, cool-water group, increased between 1975 and 1989 and declined after 1990 (Table 10). During 1975–1989, mean position moved to the north and east, followed by a return to approximately the original mean position for 1990–1994. Both latitudinal and longitudinal ranges decreased throughout 1975–1994. This group occupied waters northeast of Newfoundland prior to 1990, but was absent from this area after 1990.

Prevalence of the PC11 assemblage, the most northern, coldest-water assemblage, initially declined, but increased markedly after 1985. Mean position moved to the south and east. Variation in range was erratic, driven in part by a few atypical sites, some of which were north of the study area boundary, in areas that have been poorly sampled. Much of the recent increase in this group occurred in deep waters between Hamilton Bank and northern Grand Banks.

The analysis of temporal change in species assemblage composition and location is not comprehensive, serving mainly to illustrate the potential of the data set for such analysis. A variety of other methods and approaches should be explored to investigate this aspect of groundfish assemblage biogeography on the east coast of North America (Brown et al. 1996) and also to examine changes for individual species.

Biogeographical patterns

The dendrogram derived from clustering the 18 PCs obtained from the 1975–1994 data shows the hierarchical relationships among the species assemblages derived from the PCA, together with the aggregate distributions of sites from the PCs forming four major clusters (Fig. 6). These aggregate groups are intended to show the major biogeographic breaks in assemblage distributions. Similarly, the dendrogram showing hierarchical relationships among the site CGs, together with maps for four aggregate groups, is shown in Fig. 7, which is directly comparable with Fig. 6.

The aggregate distributions of PC and CG assemblages do not indicate clear latitudinal breaks in assemblage distributions (Figs. 6 and 7). They appear to reflect a combination of depth and latitudinal effects. For the PC assemblages, the breaks, in

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Table 7. Species assigned to each site CG, based on having their highest mean standardized catch per tow (mean) in that site cluster.

Species	Mean	Species	Mean
CGI		CG12	
None		Butterfish	9.4
		Fourspot flounder	10.6
CG2		Little skate	14.1
Lumpfish	9.7	Northern sand lance	4.5
Shorthorn sculpin	13.7	Spiny dogfish	5.5
		Windowpane	18.4
CG3		Winter skate	10.8
Arctic cod	8.5		
Atlantic sea poacher	9.3	CG13	
		Blue hake	19.8
CG4		Greenland halibut	2.6
Atlantic argentine	3.9	Longnose eel	21.0
Atlantic halibut	3.0	Northern wolffish	2.8
Haddock	4.3	Polar sculpin	3.9
Pollock	5.1	Roughhead grenadier	4.8
		Rock grenadier	25.6
CG5		Spinytail skate	4.8
Atlantic hagfish	4.5		
Marlin-spike	4.4	CG14	
White hake	2.8	Alligatorfish	11.4
		Fourbeard rockling	4.9
CG6		Moustach sculpin	12.0
Spotted wolffish	3.3	Snowflake hookear sculpin	76.8
		Smooth skate	3.2
CG7		Thorny skate	1.5
None			
		CG15	
CG8		Fawn cusk-eel	24.7
American plaice	1.9	Gulf stream flounder	14.5
Atlantic cod	1.9	Red hake	7.2
Greenland cod	55.6	Spotted hake	14.9
Snake blenny	21.0		
		CG16	
CG9		Smooth dogfish	33.6
Large-scale tapirfish	14.2		
		CG17	
CG10		Blackbelly rosefish	31.9
Black dogfish	10.0	Cusk	6.4
Longfin hake	14.0	Offshore hake	40.0
Redfishes	2.6	Shortfin squid	4.9
Roughnose grenadier	65.5	Shortnose greeneye	47.9
Witch flounder	3.6		
		CG18	
CG11		Black sea bass	62.9
Cunner	12.1	Goosefish (angler)	4.6
Longhorn sculpin	8.9	Northern searobin	29.1
Ocean pout	8.6	Scup	31.5
Sea raven	6.1	Silver hake	5.5
Winter flounder	17.0	Summer flounder	27.9
Yellowtail flounder	4.8		

Note: Catch per tow was standardized to overall species mean log catch per tow.

hierarchical order, appear to separate southern shallow assemblages occurring mainly on the southeastern Grand Banks and on shelf areas to the south (Fig. 6*d*), deep assemblages occurring mainly in the Gulf of St. Lawrence, the Gulf of Maine, and along the shelf slopes from Georges Bank to the Labrador Shelf (Fig. 6*c*), deep assemblages occurring mainly on the Labrador shelf and the Flemish Cap, but also in deep shelf areas to the south (Fig. 6*b*), and medium-depth assemblages distributed throughout the study area, but least strongly represented on the Labrador Shelf (Fig. 6*a*).

The pattern for the aggregate CG assemblages is similar (Fig. 7). The breaks, in hierarchical order, appear to separate southern shallow assemblages occurring mainly south of the Fundian Channel (Fig. 7*d*), deep assemblages occurring

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Fig. 4. Maps of selected groundfish assemblages (CGs) derived from cluster analysis of the 1975–1994 data. The species compositions shown are taken from Table 7.

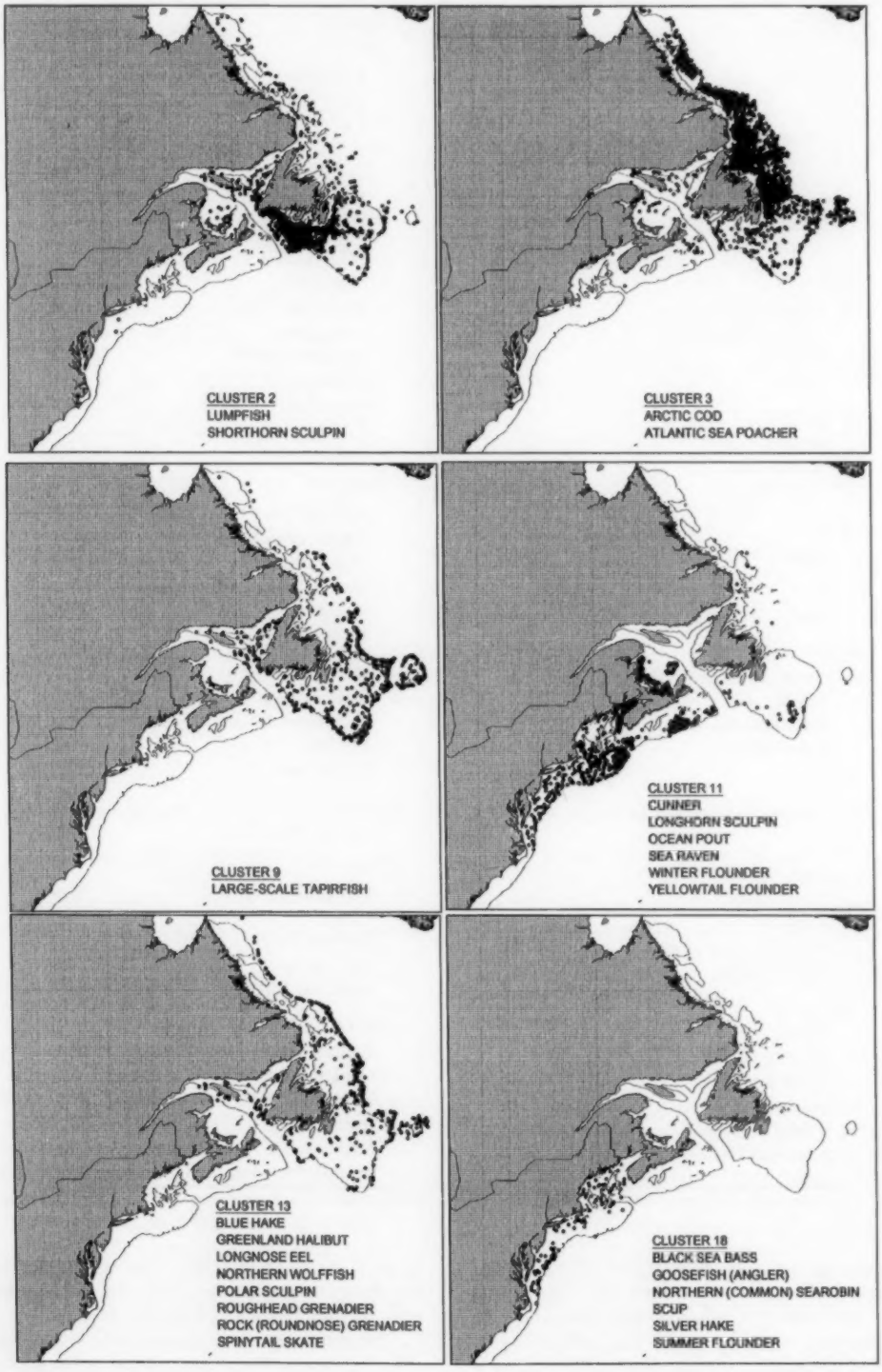

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Table 8. Mean, standard deviation, and range of bottom temperature (°C) and depth (m) for the sets included in the top 5% of the PCs and for sets in each CG.

	Bottom temperature					Depth			
	Mean	SD	Min.	Max.		Mean	SD	Min.	Max.
PC					PC				
11	1.0	1.6	−1.9	14.6	5	52	26	13	313
17	1.2	2.8	−1.7	16.2	7	68	53	13	496
8	2.3	1.4	−1.6	11.7	1	98	65	18	590
13	2.5	3.2	−1.5	18.0	18	101	98	13	835
10	3.2	2.8	−1.6	15.6	3	105	124	13	1485
16	3.4	1.9	−0.9	17.1	4	124	62	17	525
2	4.1	2.2	−1.2	19.9	13	127	82	20	695
6	5.5	1.7	−0.4	20.4	17	131	72	20	499
14	5.7	1.9	−1.5	21.7	9	159	115	13	1276
15	5.8	2.5	−1.2	19.9	15	163	125	13	960
12	6.1	2.3	−1.2	19.9	10	188	133	22	696
5	6.6	4.1	−1.6	18.4	12	230	112	15	1239
9	7.9	4.0	−1.6	23.0	11	261	108	27	889
18	8.0	5.2	−1.5	24.3	8	280	83	58	790
4	8.1	2.0	0.9	16.9	6	363	117	25	695
3	9.4	5.1	−1.2	26.8	16	371	156	18	1100
7	9.6	4.9	−1.5	24.0	14	391	187	20	1485
1	11.2	2.5	−0.8	25.9	2	537	245	20	1485
CG					CG				
2	0.7	2.3	−1.7	15.6	11	58	54	13	579
3	1.0	1.8	−1.7	10.2	12	85	67	15	382
8	1.7	2.4	−1.5	14.6	18	101	63	20	265
1	2.1	3.2	−1.5	27.1	16	111	78	20	380
6	2.5	1.9	−1.7	12.5	15	114	67	24	336
14	2.7	2.4	−1.1	11.0	7	117	76	16	457
9	3.0	2.0	−1.6	8.2	8	129	109	27	808
13	3.1	1.9	−1.4	8.4	4	133	91	13	1105
5	4.5	2.1	−1.7	14.9	14	134	93	33	578
10	5.8	1.6	−1.5	12.6	17	140	82	27	382
4	7.5	3.4	−1.6	28.0	2	151	115	27	1168
11	8.5	3.7	−1.3	23.1	1	173	136	14	1485
17	9.3	3.2	−1.2	19.9	3	255	129	37	1226
7	10.3	3.1	3.5	24.4	6	295	150	40	1375
15	10.8	3.4	3.2	24.1	5	304	127	23	1375
16	10.9	3.7	4.9	24.3	10	339	101	84	790
18	11.0	3.8	4.9	21.3	9	372	226	40	1331
12	11.9	2.9	−0.8	25.9	13	504	308	42	1432

mainly in the Gulf of St. Lawrence (Fig. 7*c*), deep assemblages occurring mainly on the slopes of the Grand Banks, Flemish Cap, and Labrador Shelf (Fig. 7*b*), and shallow to medium-depth ubiquitous assemblages (Fig. 7*a*) that appear to have a distribution similar to the group in Fig. 6*a*.

Discussion

Comparison of assemblage analyses

Given that there is subjectivity in the assemblage analyses, it is useful to compare the results of the different methods. Consistency of results among the approaches would enhance credibility, while major inconsistencies would bring the results into question.

There is a high degree of consistency among the species groupings resulting from the visual, PC, and cluster analyses (Table 11). Species occurring on the same PC or in the same CG usually fell within the same visually defined group. This indicates that the multivariate methods produced results consistent with the obvious major distribution patterns. One exception was the Atlantic halibut/haddock pair, which emerged together in all the multivariate analyses, but these species were placed in different groups during the visual classification. The probable cause of this discrepancy is that Atlantic halibut is distributed further north than haddock, but they co-occur frequently in their area of overlap.

The groups that were identified consistently by the visual classification and the multivariate analysis may be the most well-defined assemblages (Table 11). These groups are suggested as being the most appropriate for further studies focusing on species interactions as possible determinants of

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Table 9. Comparison of species groupings from PCA for all years and by 5-year periods.

All years		1975–1979		1980–1984	
PC	Species	PC	Species	PC	Species
1	**Gulf Stream flounder**	6	**Gulf Stream flounder**	2	**Fawn cusk-eel**
1	**Fourspot flounder**	6	**Fawn cusk-eel**	2	**Spotted hake**
1	**Fawn cusk-eel**	6	**Spotted hake**	2	**Gulf Stream flounder**
1	**Spotted hake**	6	**Fourspot flounder**	2	**Fourspot flounder**
1	**Butterfish**	6	**Butterfish**	2	**Butterfish**
1	Red hake			2	Shortfin squid
1	Goosefish (angler)				
2	**Blue hake**	2	**Blue hake**	1	**Blue hake**
2	**Rock (roundnose) grenadier**	2	**Rock (roundnose) grenadier**	1	**Longnose (slatjaw cutthroat) eel**
2	**Longnose (slatjaw cutthroat) eel**	2	**Longnose (slatjaw cutthroat) eel**	1	**Large-scale tapirfish**
2	**Large-scale tapirfish**	2	**Large-scale tapirfish**	1	**Rock (roundnose) grenadier**
2	**Roughhead grenadier**	2	**Roughnose grenadier**	1	Marlin-spike (common grenadier)
2	Marlin-spike (common grenadier)	2	Black dogfish	1	Roughhead grenadier
		2	Roughhead grenadier	1	Northern wolffish
3	**Scup**	7	**Black sea bass**	3	**Summer flounder**
3	**Summer flounder**	7	**Northern (common) searobin**	3	**Scup**
3	**Northern (common) searobin**	7	**Scup**	3	**Northern (common) searobin**
3	**Black sea bass**	7	Spotted hake	3	**Black sea bass**
3	**Smooth dogfish**	7	Fourspot flounder	3	**Smooth dogfish**
		13	**Smooth dogfish**	3	Windowpane
		13	**Summer flounder**		
		13	**Scup**		
4	**Silver hake**	5	**Red hake**	4	**Red hake**
4	**Red hake**	5	**Silver hake**	4	**Silver hake**
4	**Cusk**	5	**Goosefish (angler)**	4	**Ocean pout**
4	**Pollock**	5	Ocean pout	4	**Goosefish (angler)**
4	**Spiny dogfish**	5	White hake	4	Fourspot flounder
4	White hake	5	Fourspot flounder	4	Gulf Stream flounder
4	Ocean pout	14	**Pollock**	4	Longhorn sculpin
4	Goosefish (angler)	14	**Cusk**	4	Little skate
4	Haddock	14	Haddock	11	**Cusk**
4	Shortfin squid			11	**Pollock**
				11	Spiny dogfish
				11	Haddock
5	**Longhorn sculpin**	4	**Sea raven**	6	**Winter flounder**
5	**Sea raven**	4	**Longhorn sculpin**	6	**Sea raven**
5	**Yellowtail flounder**	4	**Yellowtail flounder**	6	**Longhorn sculpin**
5	**Winter flounder**	4	Winter flounder	6	**Cunner**
5	Ocean pout	4	Ocean pout	6	Yellowtail flounder
		4	Haddock	6	Ocean pout
6	**Marlin-spike (common grenadier)**	11	**Atlantic argentine**		
6	**Redfishes (*Sebastes* spp.)**	11	**White hake**		
6	**Witch flounder**	11	**Redfishes (*Sebastes* spp.)**		
6	**Black dogfish**	11	Marlin-spike (common grenadier)		
6	Atlantic argentine	11	Longfin hake		
6	White hake	11	Witch flounder		
6	Longfin hake	11	Atlantic halibut		

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1985–1989		1990–1994	
PC	Species	PC	Species
1	**Gulf Stream flounder**	4	**Gulf Stream flounder**
1	**Fourspot flounder**	4	**Fourspot flounder**
1	**Fawn cusk-eel**	4	**Fawn cusk-eel**
1	**Spotted hake**	4	**Spotted hake**
1	**Butterfish**	4	**Butterfish**
1	Red hake	4	Little skate
1	Little skate	4	Red hake
1	Goosefish (angler)		
2	**Blue hake**	1	**Blue hake**
2	**Longnose (slatjaw cutthroat) eel**	1	**Longnose (slatjaw cutthroat) eel**
2	**Rock (roundnose) grenadier**	1	**Rock (roundnose) grenadier**
2	**Large-scale tapirfish**	1	**Roughhead grenadier**
2	Roughhead grenadier	1	Large-scale tapirfish
		1	Northern wolffish
		1	Spinytail skate
		1	Greenland halibut
		1	Marlin-spike (common grenadier)
3	**Black sea bass**	3	**Black sea bass**
3	**Northern (common) searobin**	3	**Northern (common) searobin**
3	**Scup**	3	**Summer flounder**
3	**Summer flounder**	3	**Scup**
19	**Smooth dogfish**	3	**Smooth dogfish**
19	Summer flounder		
4	**Silver hake**	6	**Silver hake**
4	**Red hake**	6	**Red hake**
4	**Spiny dogfish**	6	**Goosefish (angler)**
4	**Cusk**	6	**White hake**
4	Ocean pout	6	Ocean pout
4	Pollock	6	Spiny dogfish
4	Shortfin squid	9	**Cusk**
4	White hake	9	**Pollock**
		9	Atlantic argentine
		9	Spiny dogfish
5	**Longhorn sculpin**	5	**Longhorn sculpin**
5	**Winter flounder**	5	**Sea raven**
5	**Sea raven**	5	**Yellowtail flounder**
5	**Yellowtail flounder**	5	**Winter flounder**
5	Ocean pout	5	Ocean pout
5	Alligatorfish	5	Haddock
5	Winter skate		
6	**Atlantic argentine**	2	**Black dogfish**
6	**Redfishes (*Sebastes* spp.)**	2	**Marlin-spike (common grenadier)**
6	**White hake**	2	**Longfin hake**
6	**Marlin-spike (common grenadier)**	2	**Redfishes (*Sebastes* spp.)**
6	Pollock	2	**Witch flounder**
6	Witch flounder	2	White hake
6	Goosefish (angler)	2	Greenland halibut
7	**Roughnose grenadier**		
7	**Longfin hake**		
7	**Black dogfish**		
7	Witch flounder		
7	Redfishes (*Sebastes* spp.)		

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Table 9 (*continued*).

All years		1975–1979		1980–1984	
PC	Species	PC	Species	PC	Species
7	**Windowpane**	1	**Little skate**	5	**Winter skate**
7	**Winter skate**	1	**Windowpane**	5	**Windowpane**
7	**Little skate**	1	**Winter skate**	5	**Little skate**
7	Northern sand lance	1	**Spiny dogfish**	5	Spiny dogfish
		1	Winter flounder		
		1	Longhorn sculpin		
		1	Northern sand lance		
		1	Red hake		
		1	Fourspot flounder		
8	**Spotted wolffish**	8	**Atlantic wolffish**	8	**Atlantic wolffish**
8	**Atlantic wolffish**	8	**Spotted wolffish**	8	**Spotted wolffish**
8	**Northern wolffish**	8	**Atlantic cod**	8	**Northern wolffish**
8	Roughhead grenadier	8	**Northern wolffish**	8	**Atlantic cod**
8	Atlantic cod			8	Greenland halibut
8	Greenland halibut				
8	Redfishes (*Sebastes* spp.)				
9	**Blackbelly rosefish**	12	**Blackbelly rosefish**	9	**Blackbelly rosefish**
9	**Offshore hake**	12	**Offshore hake**	9	**Offshore hake**
9	**Shortnose greeneye**	12	**Shortnose greeneye**	9	**Shortnose greeneye**
10	**Thorny skate**	15	**American plaice**	12	**Thorny skate**
10	**American plaice**	15	**Thorny skate**	12	**Yellowtail flounder**
10	Witch flounder	15	Witch flounder	12	**American plaice**
10	Atlantic cod	15	Atlantic cod	12	Northern sand lance
10	Smooth skate				
11	**Arctic cod**	9	**Arctic cod**	14	**Arctic cod**
11	**Atlantic sea poacher**	9	**Atlantic sea poacher**	14	**Atlantic sea poacher**
11	Greenland halibut	9	**Greenland cod**	14	Greenland halibut
11	Polar sculpin	9	Shorthorn sculpin	14	Greenland cod
		9	Greenland halibut	14	Northern wolffish
		9	Northern wolffish		
12	**Fourbeard rockling**	10	**Atlantic hagfish**	15	**Fourbeard rockling**
12	**Atlantic hagfish**	10	**Fourbeard rockling**	15	**Atlantic hagfish**
12	Smooth skate	10	**Black dogfish**	15	Marlin-spike (common grenadier)
		10	**Marlin-spike (common grenadier)**	15	White hake
		10	Witch flounder		
		10	Redfishes (*Sebastes* spp.)		
13	**Moustache (mailed) sculpin**	16	**Moustache (mailed) sculpin**	16	**Moustache (mailed) sculpin**
13	**Alligatorfish**	16	**Alligatorfish**	16	**Alligatorfish**
13	**Snowflake hookear sculpin**	17	**Smooth skate**	19	**Snowflake hookear sculpin**
		17	**Snowflake hookear sculpin**		
		17	Polar sculpin		
14	**Roughnose grenadier**			10	**Roughnose grenadier**
14	**Longfin hake**			10	**Black dogfish**
14	**Black dogfish**			10	**Longfin hake**
				10	Witch flounder
15	**Atlantic halibut**			13	**Atlantic halibut**
15	**Haddock**			13	Atlantic argentine
				13	White hake
				13	Redfishes (*Sebastes* spp.)
				13	Haddock
				13	Marlin-spike (common grenadier)
				13	Goosefish (angler)

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1985–1989		1990–1994	
PC	Species	PC	Species
8	**Winter skate**	7	**Windowpane**
8	**Little skate**	7	**Winter skate**
8	**Windowpane**	7	**Little skate**
8	**Northern sand lance**	7	Fourspot flounder
		19	**Northern sand lance**
10	**Spotted wolffish**	11	**Atlantic wolffish**
10	**Atlantic wolffish**	11	**Spotted wolffish**
10	**Northern wolffish**	11	Northern wolffish
10	Atlantic cod		
10	Roughhead grenadier		
10	Greenland halibut		
11	**Offshore hake**	10	**Shortnose greeneye**
11	**Blackbelly rosefish**	10	**Offshore hake**
11	**Shortnose greeneye**	10	**Blackbelly rosefish**
12	**Thorny skate**	12	**Thorny skate**
12	**American plaice**	12	**American plaice**
12	Atlantic cod	12	Smooth skate
12	Witch flounder	12	Atlantic cod
12	Shortfin squid	12	Witch flounder
15	**Arctic cod**	13	**Atlantic sea poacher**
15	**Atlantic sea poacher**	13	**Arctic cod**
15	Greenland cod	13	Greenland halibut
15	Greenland halibut		
9	**Fourbeard rockling**	14	**Atlantic hagfish**
9	**Atlantic hagfish**	14	**Fourbeard rockling**
9	**Smooth skate**	14	Smooth skate
9	Marlin-spike (common grenadier)	14	Snowflake hookear sculpin
9	White hake		
9	Redfishes (*Sebastes* spp.)		
13	**Moustache (mailed) sculpin**	8	**Moustache (mailed) sculpin**
13	**Snowflake hookear sculpin**	8	**Alligatorfish**
13	**Alligatorfish**	8	**Greenland cod**
		8	**Snowflake hookear sculpin**
		8	Snake blenny
7	**Roughnose grenadier**	18	**Roughnose grenadier**
7	**Longfin hake**	2	**Black dogfish**
7	**Black dogfish**	2	**Marlin-spike (common grenadier)**
7	**Witch flounder**	2	**Longfin hake**
7	Redfishes (*Sebastes* spp.)	2	**Redfishes (*Sebastes* spp.)**
		2	**Witch flounder**
		2	White hake
		2	Greenland halibut
16	**Atlantic halibut**	15	**Atlantic halibut**
16	**Haddock**	15	**Haddock**

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Table 9 (*concluded*).

All years		1975–1979		1980–1984	
PC	Species	PC	Species	PC	Species
16	**Polar sculpin**	3	**Roughhead grenadier**	7	**Witch flounder**
16	Roughhead grenadier	3	**Spinytail skate**	7	**Greenland halibut**
16	Greenland halibut	3	**Greenland halibut**	7	**Smooth skate**
		3	**Northern wolffish**	7	**Roughhead grenadier**
		3	Redfishes (*Sebastes* spp.)	7	**Polar sculpin**
		3	Polar sculpin	7	**Spinytail skate**
		3	Marlin-spike (common grenadier)	7	Redfishes (*Sebastes* spp.)
		3	Large-scale tapirfish		
17	**Lumpfish**	18	Atlantic halibut	17	**Lumpfish**
17	**Shorthorn sculpin**	18	**Lumpfish (—)**	17	**Shorthorn sculpin**
17	Sea raven	18	Shorthorn sculpin (—)	17	Sea raven
18	**Cunner**	19	**Cunner**	6	**Winter flounder**
18	Winter flounder	19	Snake blenny	6	**Sea raven**
18	Snake blenny			6	**Longhorn sculpin**
				6	**Cunner**
				6	Yellowtail flounder

Note: Groups are determined by species scores on the PCs. Species in bold type have loadings >0.5; species in standard type have loadings <0.5 and >0.3. Assemblages from the 5-year time periods are grouped with the overall time period assemblages that they most closely resemble.

Table 10. Comparisons of the numbers of points and mean positions (latitude and longitude) for sites scoring in the top 5% for PCs 10 and 11 by 5-year time period.

			Latitude			Longitude		
PC	Time period	# of points	Mean	SD	Range	Mean	SD	Range
PC10	1975–79	414	46.2	3.1	20.1	–57.4	6.6	34.0
	1980–84	476	46.9	3.2	16.0	–56.4	6.5	27.5
	1985–89	598	47.0	2.6	18.0	–55.1	6.2	27.5
	1990–94	446	46.2	2.3	12.8	–57.1	5.9	24.6
			$P < 0.0001$			$P < 0.05$		
PC11	1975–79	287	54.8	4.3	24.9	–57.2	3.5	22.6
	1980–84	233	52.5	3.1	14.3	–55.1	2.6	16.9
	1985–89	631	53.8	5.5	24.8	–56.0	3.3	17.2
	1990–94	783	51.1	3.0	16.6	–54.1	3.6	18.4
			$P < 0.0001$			$P < 0.0001$		

Note: *P*-values are for comparing means using one-way analysis of variance.

assemblage structure. There are a few instances of consistently occurring groups of four to five species, e.g., smooth skate/ thorny skate/Atlantic cod/American plaice in the North-temperate Bank/Slope group and summer flounder/scup/northern searobin/ smooth dogfish/black sea bass in the Southern Bank/Slope group. There are also many smaller groups, particularly pairs, of species that appear to co-occur consistently. For example, the Southern Deepwater group of blackbelly rosefish/offshore hake/shortnose greeneye was identified in all analyses and appears to have a distinctive distribution.

The correspondence between PCA site groups (top 5% of sites on each PC) and CGs is variable (Fig. 8). In some cases, e.g., PC17 with CG2 and PC11 with CG3, the correspondence is nearly one-to-one. In other cases, such as PCs 1, 3, and 4, the sites are distributed over several CGs. For each PC, the chi-square statistic was used to test whether the distribution of sites in the CGs was significantly different from random, based on the total numbers of sites within each site CG. In each case the difference from a random distribution was significant ($P < 0.01$). Therefore, it is evident that PC site groups tend to correspond to particular site CGs, as would be expected if both methods were perceiving similar assemblage distributions, but that the strength of this correspondence varies.

The geographic correspondence between PCs and CGs is reflected in the distribution plots for the individual PCs and CGs (Figs. 3 and 4). The distribution of sites for PC17 and CG2, which both represent the lumpfish/shorthorn sculpin species pair, is very similar. PC2 and CGs 9 and 13 also correspond well, but there are many shallow sites in the CGs that are not seen in PC2. In contrast, PC4, which represents the South-temperate Bank/Slope group silver hake/red hake/cusk/ pollock/spiny dogfish/white hake/ocean pout, goosefish (angler)/haddock/shortfin squid, is distributed over several CGs.

The PCA and site cluster analysis both provided information

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1985–1989		1990–1994	
PC	Species	PC	Species
14	**Polar sculpin**	1	**Blue hake**
14	**Greenland halibut**	1	**Longnose (slatjaw cutthroat) eel**
14	Roughhead grenadier	1	**Rock (roundnose) grenadier**
		1	**Roughhead grenadier**
		1	Large-scale tapirfish
		1	**Northern wolffish**
		1	Spinytail skate
		1	Greenland halibut
		1	Marlin-spike (common grenadier)
17	**Lumpfish**	16	**Lumpfish**
17	**Shorthorn sculpin**	16	**Shorthorn sculpin**
17	Sea raven	16	Sea raven
18	**Cunner**	17	**Cunner**
18	Winter flounder	17	Butterfish
18	Snake blenny		

on the strength of species affinities for their assemblages. There is moderate agreement between the two approaches, in that the maximum species loadings on the PCs (Table 4) are correlated with log-transformed maximum relative abundances (Table 7) ($r = 0.48$, $P < 0.01$).

Summary descriptions of the assemblages, based on their geographical, depth, and temperature distributions, indicate that depth and temperature play important roles in determining assemblage distributions (Table 12). This is consistent with the findings of several previous studies (Overholtz and Tyler 1985; Mahon and Smith 1989*a*; Gabriel 1992; Gomes et al. 1995).

The PCA revealed patterns of species association broadly consistent with previous studies of smaller regions (Table 13). Comparing the present study with previous, more localized studies is subjective owing to the different methodologies and species used. No study has yet defined assemblages for the northern Labrador Shelf. The assemblages found in the present study were most consistent with those reported by Gabriel (1992) for the area from Cape Hatteras north to the Scotian Shelf and by Mahon (1997) for the Scotian Shelf and Bay of Fundy. The approach to assemblage definition taken by Gomes et al. (1992) and Guadalupe Villagarcia (1995) emphasized the absolute abundance of species in an assemblage, so their results are difficult to compare with those of this study. However, the combination of thorny skate, American plaice, and Atlantic cod also emerged as a dominant group in their analyses.

The finding of a large number of sites without assemblages has not been reported by previous studies in the area (e.g., Overholtz and Tyler 1985; Gabriel 1992; Gomes et al. 1995; Mahon 1997). However, the similarity of the results for the PCA and site cluster analysis in this study supports this interpretation for our data set. For the PCA, 45.5% (17 612) of the sampled sites do not score in the top 5% of any PC. For the cluster analysis, 42.6% (16 474) of the sites are in the first cluster, which is interpreted as not having any characteristic assemblage. A total of 7823 (i.e., somewhat less than half) of these are the same sites, which suggests that these two methods differed somewhat in determining which sites were not assignable to an assemblage. This discrepancy might be reduced with other distance indices or clustering methods, or by modifying the top 5% criterion for inclusion of a site in a PCA-defined assemblage.

Several factors could have contributed to this apparent lack of structure. The large spatial scale of the data set could mask relationships that exist in smaller regions or that vary spatially. Some of the lack of structure in the results could be due to the sampling gear, which integrates species occurring over a transect of 1.8 nautical miles, and may, therefore, integrate assemblages that do not occur in the same habitat. It could also be due to the use of data obtained from different sources, which used different trawling gear and vessels. Using data from several months may also have been a factor. Seasonal differences in assemblage structure have been described in several studies (e.g., Colvocoresses and Musick 1984; Mahon and Smith 1989*a*). Assemblages appear to be stable in winter and summer and, due to seasonal migrations, to be transitional in spring and fall (Musick et al. 1985; Phoel 1985).

Although analyzing the 20-year time period covered in the present data set might have obscured relationships that occurred over shorter time periods, the similarity between the PCAs for the 1975–1994 period and the 5-year periods suggests that the temporal span of the data set was not a major factor in limiting assemblage structure.

The apparent lack of structure could also be a reflection of the real nature of the demersal fish assemblages, which may not be highly structured into functional groups. The mobility of most fish species, and their relatively large ranges, together with the migratory habits of many species, may preclude

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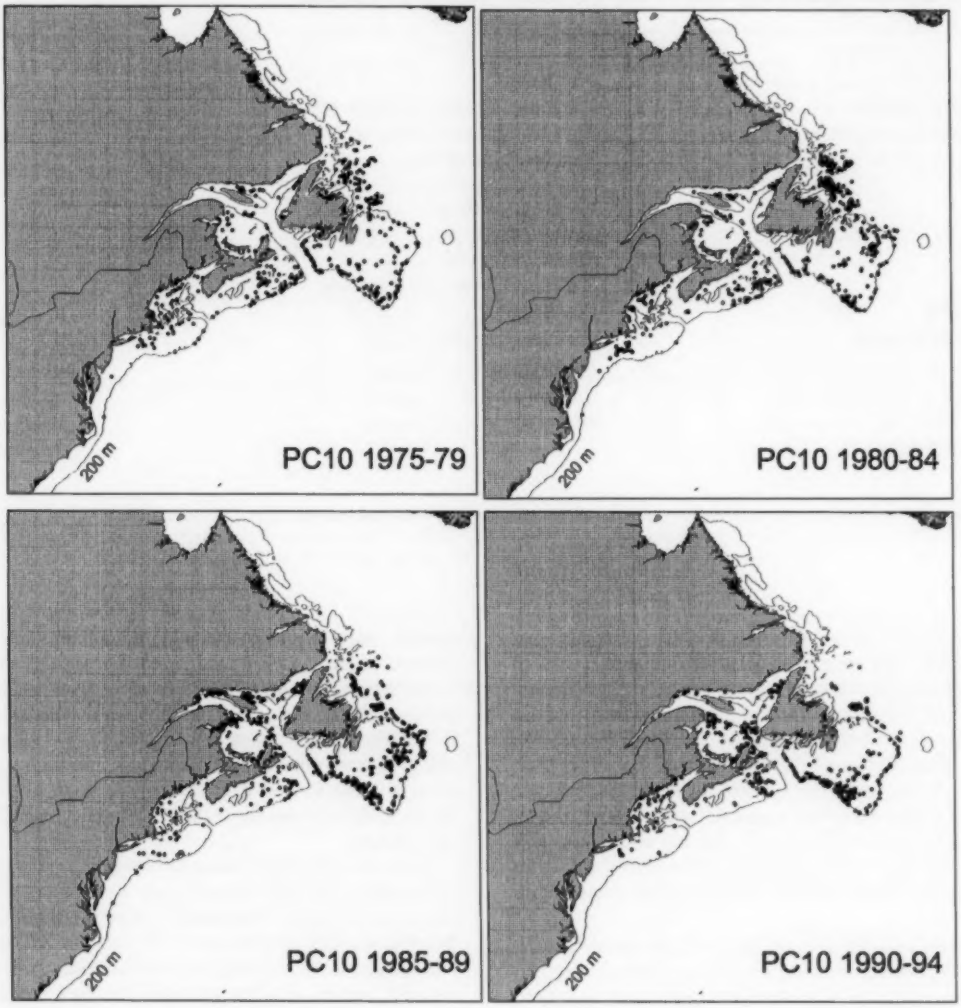
Fig. 5. Temporal shifts in the distribution of sites for PCs 10 and 11, based on the top 5% of scores for the PCs in 5-year intervals.



development of tight functional relationships among groups of more than two or three species. Because fishes change their ecological roles as they grow, and size was not considered in the analyses conducted for this paper, functional relationships among size-classes of various species may have been masked. In addition, some naturally occurring functional assemblages may have been disrupted by fishing mortality.

Biogeography

The east coast of North America is generally considered as comprising five biogeographical provinces: Arctic, Nova Scotian, Virginian, Carolinian, and Caribbean (Hayden and Dolan 1976). The boundaries between these provinces are considered to be at 47°N, about Cape Race, Newfoundland, 41°N, about Cape Cod, Massachusetts, 35°N, about Cape Hatteras, North Carolina, and 30°N, just below Jacksonville, Florida. Hazel (1970) separated the Arctic Province into Arctic and Labrador provinces at about the Hudson Strait. Thus, according to the literature, there should be areas of faunal discontinuity at two boundaries that fall within the area of the present study: approximately from Cape Race across the northern Grand Banks and in the vicinity of Cape Cod.

These boundaries or transition zones have been defined primarily with regard to the distribution of nearshore marine fauna and flora (Briggs 1974). Regarding fishes, McAllister (1960) identified Battle Harbour, Labrador, just north of the Strait of Belle Isle, as the area of transition from the Arctic (Labrador) to the Atlantic faunas. There does not appear to have been any comprehensive analysis of fish distributions throughout the study area with a view to evaluating the extent to which fishes fit the generally accepted biogeographical patterns. Although the biogeographical literature does not provide a clear definition of where a Cape Cod boundary would extend across the shelf, it would most likely be at Nantucket Shoals

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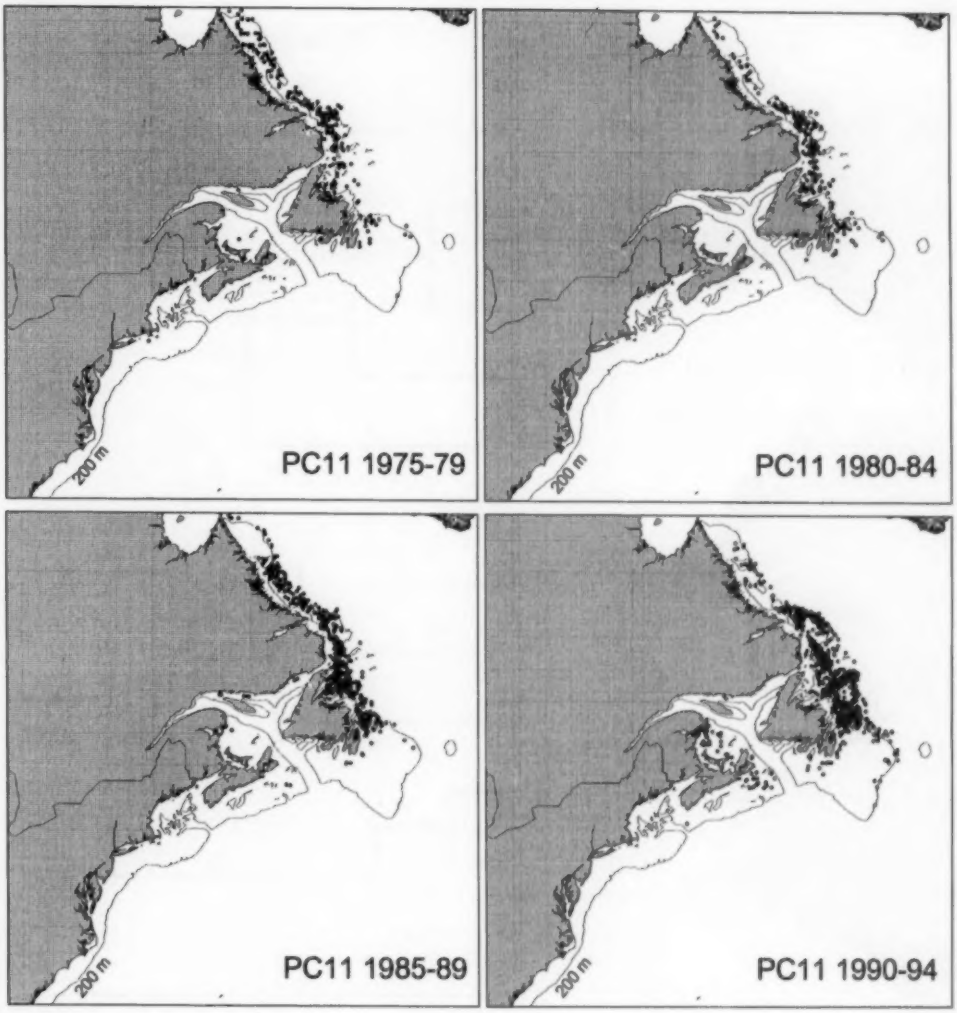
Fig. 5 (*concluded*).



due south of the cape (Phoel 1985). For fishes, the boundary was seasonal, functioning in summer, but not in winter (Phoel 1985). The present study does not attempt a comprehensive biogeographic analysis, but does provide insights that indicate the need for this issue to be addressed in greater detail, a purpose for which the present data set is ideally suited.

Only one other study has examined the entire study area (Mahon and Sandeman 1985). That study, which aimed at investigating broad biogeographical patterns, analyzed 1970–1980 trawl survey data from Cape Hatteras, North Carolina, to Cape Chidley, Labrador, by aggregating trawl sets into bands about 30 nautical miles in width for two depth zones: 50–200 and >200 m. These depth zones were assumed to represent shelf and slope fishes, respectively. The bands were then clustered on the basis of the species that occurred in them. That approach imposed a limit of 30 nautical miles on the spatial scale at which distribution patterns could be resolved. The resolution of biogeographical patterns was also constrained by the orientation of the bands, which was approximately perpendicular to the coastline. Breaks in distribution for fishes occurred in both depth zones in the areas of Cape Cod and the tail of the Grand Banks. For fishes at depths of 50–200 m, there was a break on the southwest Scotian Shelf whereas for fishes occurring deeper than 200 m, there was break at about the middle of the Scotian Shelf.

There are no obvious faunal boundaries in the Cape Cod area, the Cape Race area, or the mid-Scotian Shelf in either the PC or CG analyses of the present study. The major break in the PC assemblages does suggest a group of assemblages that is seldom present north of the latitude of Cape Race (Fig. 6). In contrast, most of the predominantly northern assemblage groups extend south of that area. Only PC assemblages 8, 11, and 16 and CG assemblage 3 appear to have southern boundaries in the Cape Race area (Figs. 3 and 4). No PC or CG

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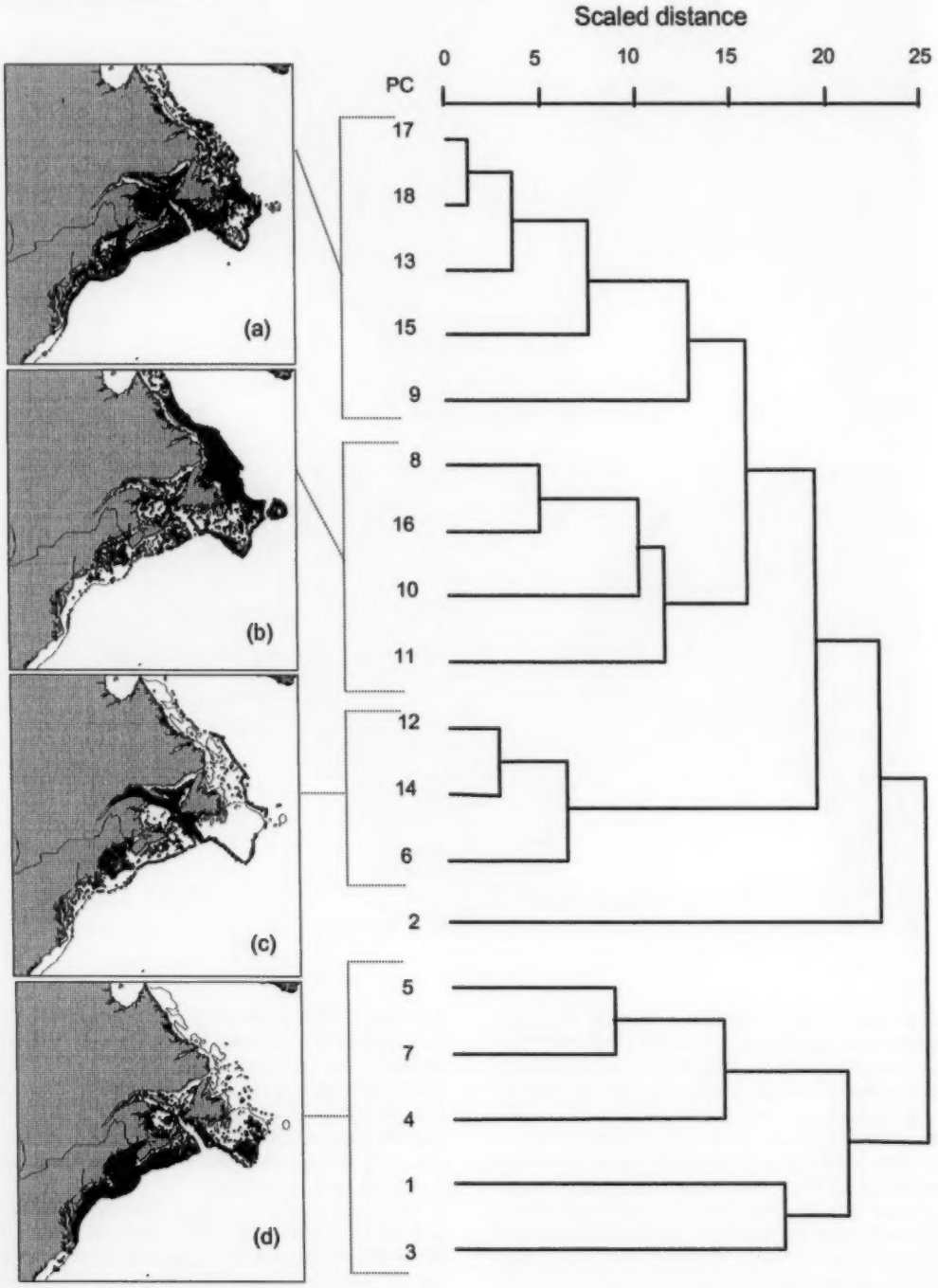
Fig. 6. Dendrogram of hierarchical relationships among the groundfish assemblages derived from PCA, with maps for four aggregate groups (the map for PC2 can be seen in Fig. 3).



assemblages have boundaries in the Cape Cod area (i.e., not extending out onto Georges Bank).

It appears that the assemblage distribution patterns are considerably more complex than can be accounted for by the standard two-dimensional approach to defining biogeographical boundaries. Depth plays an important role in structuring demersal fish assemblages, and the boundaries of assemblages at different depths do not always coincide. Temperature is also

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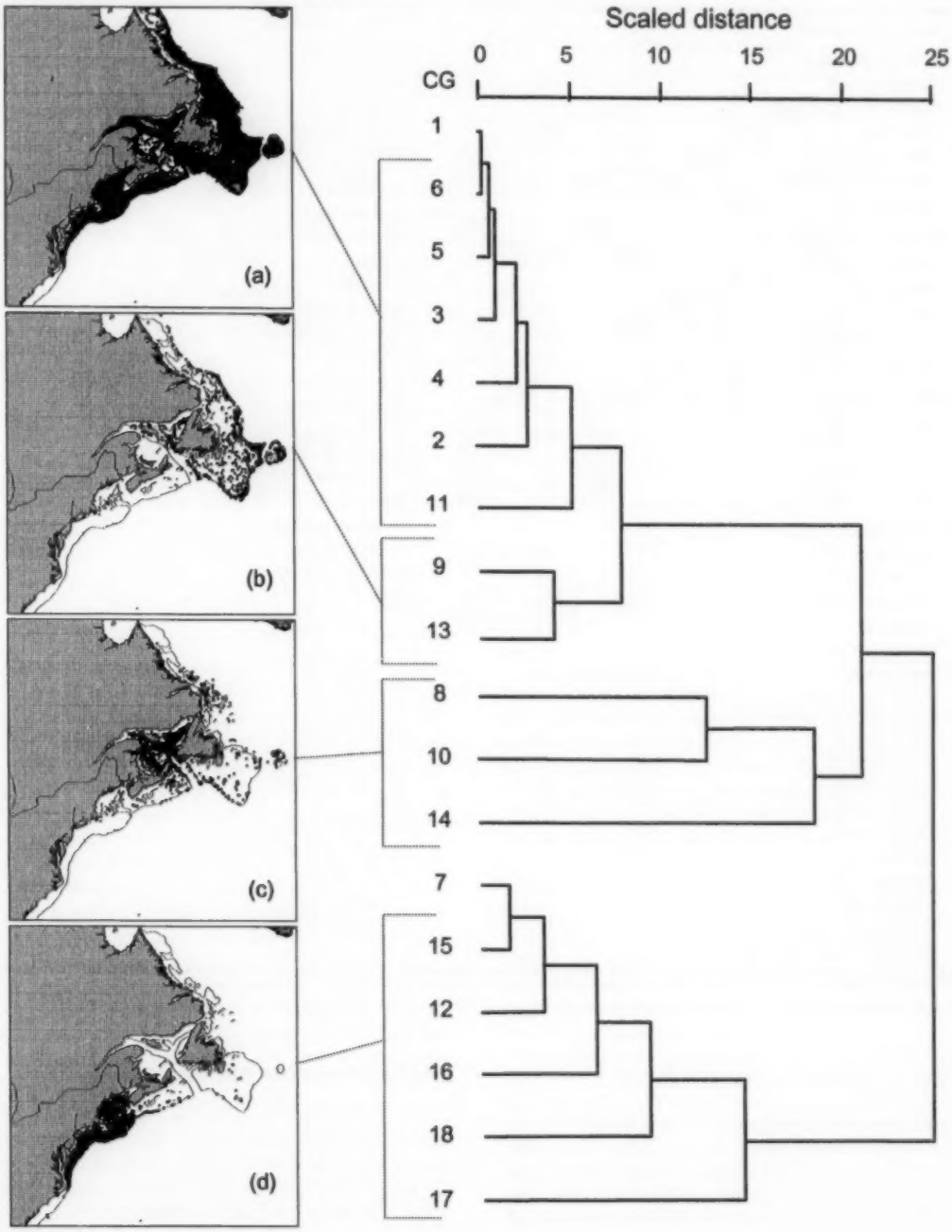
Fig. 7. Dendrogram of hierarchical relationships among CGs, with maps for four aggregate groups (CGs 1 and 7 were not included in the maps, as no assemblages were associated with these groups).



an important determinant of distribution and can vary through time on a decadal or longer scale, as well as seasonally. Interannual shifts in temperature have been shown to result in shifts in the distribution of several of the species in the present study area (Murawski 1993).

In further examining biogeographic patterns in the study area, it will be necessary to take account of the three invariant physical dimensions, latitude, longitude, and depth, with consideration of the changes that may occur in a fourth dimension, time. This will require more detailed analysis of the codistribution patterns of individual species, including analysis of species range end-points, in relation to environmental

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Table 11. Comparisons among species groups determined by visual analysis with groups determined by the multivariate analyses.

Visual species group	PC	CG
Northern Deepwater		
Longnose eel	2	13
Largescale tapirfish	2	9
Blue hake	2	13
Rock grenadier	2	13
Roughhead grenadier	2	13
Spinytail skate	—	13
Temperate Deepwater		
Atlantic argentine	6	4
Marlin-spike	6	5
Black dogfish	6	10
Longfin hake	14	10
Roughnose grenadier	14	10
Southern Deepwater		
Shortfin squid	4	17
Blackbelly rosefish	9	17
Offshore hake	9	17
Shortnose greeneye	9	17
Northern Bank/Slope		
Northern wolffish	8	13
Spotted wolffish	8	6
Atlantic sea poacher	11	3
Arctic cod	11	3
Greenland halibut	11	13
Polar scuplin	16	13
North-temperate Bank/slope		
Redfishes	6	10
Witch flounder	6	10
Atlantic wolffish	8	14
Smooth skate	10	14
Thorny skate	10	14
Atlantic cod	10	8
American plaice	10	8
Alligator fish	13	14
Moustache sculpin	13	14
Snowflake hookear sculpin	13	14
Atlantic halibut	15	4
Lumpfish	17	2
Shorthorn sculpin	17	2
Snake blenny	18	8
Cunner	18	11
South-temperate Bank/Slope		
Goosefish (Angler)	4	18
Silver hake	4	18
Cusk	4	17
Red hake	4	15
Spiny dogfish	4	12
White hake	4	5
Pollock	4	4
Yellowtail flounder	5	11
Winter flounder	5	11
Ocean pout	5	11
Sea raven	5	11
Longhorn sculpin	5	11
Winter skate	7	12
Northern sandlance	7	12
Atlantic hagfish	12	5
Fourbeard rockling	12	14
Haddock	15	4
Southern Bank/Slope		
Fourspot flounder	1	12
Butterfish	1	12
Spotted hake	1	15
Fawn cusk-eel	1	15
Gulfstream flounder	1	15
Summer flounder	3	18
Scup	3	18
Northern searobin	3	18
Smooth dogfish	3	16
Black seabass	3	18
Windowpane	7	12
Little skate	7	12

Note: The numbers in the columns show on which species group the species had its highest loading in the PCA (PCs) (Table 6) and the grouping of species based on maximum relative abundance in the site CGs (Table 7). Lines separate species loading on different PCs.

characteristics at a finer spatial scale than that used by Mahon and Sandeman (1985).

Pinhorn and Halliday (1997) provided an example of such an analysis for slope fishes with a focus on the tail of the Grand Banks as a possible area of faunal discontinuity. They found evidence for discontinuity in faunal distributions there, but noted that whereas some species were distributed mainly north of this area, and others mainly south of it, several species were distributed widely on both sides. Their study indicated that both occurrence and density may also be useful as indicators of faunal discontinuity. Their findings support our view that when the data are examined at the level of resolution of the individual trawl sets over a wide geographical area, the emergent patterns are too complex to explain using the biogeographical generalizations that have been offered previously.

Implications for management

The analyses described in this paper may have some implications for fishery management. However, the present results are too preliminary to warrant an in-depth discussion.

One primary question that could be addressed through detailed assemblage analysis is whether functional groupings exist that can be viewed as ecological entities, such as the assemblage production units of Tyler et al. (1982). If such units exist, management strategies could be adapted to optimize harvest of assemblages, rather than harvest of single species. The analyses conducted to date indicate that the groundfish assemblages are rather loose, in that the assemblages explain only about 56% of the variance in total species distribution.

The apparent looseness of assemblage structure for the

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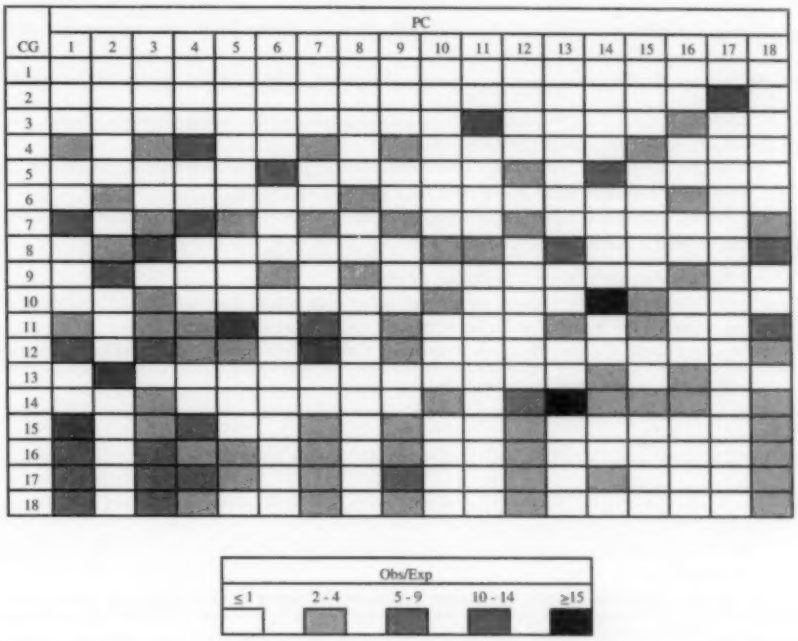
Fig. 8. Correspondence between sites in the PCA site groups and those in the site CGs. Shading indicates correspondence between sites occurring in a given PC group and site CG as determined by the ratio of observed to expected numbers of sites co-occurring in the PC and site CG pairs under a null hypothesis of no relationship.



demersal fishes of the study area suggests that, in the context of all 66 species and the entire Cape Chidley – Cape Hatteras region, the assemblages may not have strong functional relationships. The apparent persistence of assemblage structure over the study period, despite substantial fluctuations in biomass of major species throughout the study area due to fishing, further suggests that functional relationships among species may be weak, or at least adaptable.

These findings indicate that assemblages are not sufficiently coherent to support a management approach based on functionally integrated multispecies ecological units. Single-species management approaches may be more appropriate for these fisheries than would be the case for highly structured assemblages with strong functional relationships. Additional analysis (e.g., focusing on trophic studies and habitat associations within assemblages) will be required to determine whether or not some groups have functional relationships within this overall framework.

The large marine ecosystem (LME) concept has emerged in the past decade as a possible context for marine ecosystem management (Sherman and Alexander 1986). The "criteria used to geographically delimit LMEs are consideration of distinct bathymetry, hydrography, productivity, and trophically dependent populations" (Sherman 1993). According to Sherman (1993), three LMEs have been defined in the present study area: (*i*) "Northeast U.S. Continental Shelf," extending from Cape Hatteras to the Gulf of Maine and Georges Bank inclusive, (*ii*) "Scotian Shelf," including the Bay of Fundy and the southern Gulf of St. Lawrence, and (*iii*) "Newfoundland Shelf," including the Grand Banks, northern and eastern Gulf of St. Lawrence, and the Labrador Shelf to Cape Chidley.

The boundaries shown between the three areas were preliminary (Sherman 1993). Only the Northeast U.S. Continental Shelf area has received further attention as an LME (Sherman et al. 1996). However, as presented, bathymetry has clearly been a major factor in locating these boundaries. The two northern LMEs appear to be separated by the Laurentian Channel, while the Fundian Channel and deep areas of the Gulf of Maine appear to be the boundary between the two southern LMEs.

The findings of this study suggest that these two channels are not important boundaries for the distribution of demersal fishes. This is particularly true of the most abundant fishes that support the major groundfish fisheries. If the LME approach is pursued as a context for fisheries or ecosystem management in these areas, the validity of the boundaries should be evaluated using the present data set.

The present data set contains much information on bycatch species of no present commercial value. The impact of fishing on these species is little known, but is of increasing concern to conservationists, particularly since the United Nations Conference on Environment and Development (Burke et al. 1992) brought biodiversity conservation to the fore. There is increasing pressure to establish marine protected areas globally (Kelleher et al. 1996) The present data set can provide information on the distribution and persistence of areas of high fish biodiversity that could be of considerable value in identifying the best localities for protection.

Acknowledgments

This paper is the result of a collaboration between two

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Table 12. Summaries of characteristics of species assemblages derived from PCA, with associated site CGs having a ratio ≥5 in Fig. 8.

PC	CG	Characteristics
1	12, **15**, 16, 17, 18	Southern, very warm, shallow–middle depths, aggregated The most southern assemblage, restricted to the Middle Atlantic Bight and southern Georges Bank at shallow–middle depths in warm water PC species: **Gulf Stream flounder**, **fourspot flounder**, **fawn cusk-eel**, **spotted hake**, **butterfish**, red hake, goosefish (angler)
2	9, **13**	North, cold, very deep, aggregated The deepest assemblage occurring in the north, through the Laurentian channel, around the slope of the Grand Banks and Labrador Shelf and around the Flemish Cap PC species: **blue hake**, **rock (roundnose) grenadier**, **longnose (slatjaw cutthroat) eel**, **large-scale tapirfish**, **roughhead grenadier**, marlin-spike (common grenadier)
3	7, 12, **16**, 17, **18**	Southern, warm, shallow–middle depths, dispersed Primarily a southern assemblage, most commonly occurring on Georges Bank, in the Middle Atlantic Bight, and in the mouth of the Bay of Fundy, but with scattered sites up to the Grand Banks PC species: **scup, summer flounder**, **northern (common) searobin**, **black sea bass**, **smooth dogfish**
4	**4**, **7**, **15**, **17**	South-central, warm, shallow–middle depths, aggregated Concentrated in the Gulf of Maine, on the southern Scotian Shelf, and outer edges of Georges Bank and the Middle Atlantic Bight PC species: **silver hake**, **red hake**, **cusk**, **pollock**, **spiny dogfish**, white hake, ocean pout, goosefish (angler), haddock, shortfin squid
5	**11**	Central, medium temperature, very shallow, fragmented Shallowest assemblage, occurring on bank tops and some coastal areas in the middle of the study range (fragmented distribution) PC species: **longhorn sculpin**, **sea raven**, **yellowtail flounder**, **winter flounder**, ocean pout
6	**5**	Central, medium temperature, deep, localized Primarily in the Laurentian Channel, into the mouth of the St. Lawrence River PC species: **marlin-spike (common grenadier)**, **redfishes (*Sebastes* spp.)**, **witch flounder**, **black dogfish**, Atlantic argentine, white hake, longfin hake
7	11, **12**	Southern (Georges Bank), very warm, shallow, aggregated Occurs most strongly on Georges Bank, and to a lesser extent on other banks PC species: **windowpane**, **winter skate**, **little skate**, northern sand lance
8		Northern, cold, deep, aggregated Occurs on the northeastern Newfoundland and southern Labrador shelves PC species: **spotted wolffish**, **Atlantic wolffish**, **northern wolffish**, roughhead grenadier, Atlantic cod, Greenland halibut, redfishes (*Sebastes* spp.)
9	**17**	Southern widespread, warm, medium depths Occurs in the deeper on shelf areas of the Scotian Shelf and Gulf of Maine and along the shelf slope from the southwestern Grand Banks to Cape Hatteras PC species: **blackbelly rosefish**, **offshore hake**, **shortnose greeneye**
10		North-central, cool, medium-deep depths, disaggregated Occurring on the northeastern Newfoundland Shelf, Grand Banks, northern Scotian Shelf, and in the Gulf of St. Lawrence PC species: **thorny skate**, **American plaice**, witch flounder, Atlantic cod, smooth skate
11	**10**	Northern, cold, deep, aggregated Coldest water assemblage, occurs on the northeastern Newfoundland and Labrador shelves PC species: **Arctic cod**, **Atlantic sea poacher**, Greenland halibut, polar sculpin
12	**14**	Central, medium temperature, medium depth Occurs in the northern Gulf of St. Lawrence and Gulf of Maine PC species: **fourbead rockling**, **Atlantic hagfish**, smooth skate
13	8, **14**	Central, cool, shallow, scattered aggregations Occurs at several scattered localities north of Cape Cod PC species: **moustache (mailed) sculpin**, **alligatorfish**, **snowflake hookear sculpin**

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Table 12 (*concluded*).

PC	CG	Characteristics
14	5, **10**	Central, medium temperature, deep, localized Concentrated in the Laurentian Channel and in the Gulf of St. Lawrence up to the mouth of the St. Lawrence River and also along the slope of the Labrador and Scotian shelves PC species: **roughnose grenadier**, **longfin hake**, **black dogfish**
15		Central, medium temperature, medium depths Occurs mainly on the outer banks of the Scotian Shelf and on the southwestern edge of the Grand Banks into the northeastern Gulf of St. Lawrence PC species: **Atlantic halibut**, **haddock**
16		Northern, cool, deep Essentially a single-species PC concentrated on northeastern Newfoundland and Labrador slope, scattered into the Gulf of St. Lawrence, Gulf of Maine, and Georges Bank PC species: **polar sculpin**, roughhead grenadier, Greenland halibut
17	**14**	North-central, very cold, medium depths Localized on the shelf south of Newfoundland, around into the northeastern Gulf of St. Lawrence PC species: **lumpfish**, **shorthorn sculpin**, sea raven
18	8, **11**	Southern, warm, shallow Essentially a single-species PC, occurs in the southwestern Gulf of St. Lawrence, Bay of Fundy, Georges Bank, and Middle Atlantic Bight PC species: **cunner**, winter flounder, snake blenny

Note: The highest association of each site CG with a PC (in Fig. 8) is in bold type (see Table 8 for depth and temperature information). In the PC species lists, species with loadings >0.5 are in bold type; species with loadings between 0.3 and 0.5 are in standard type.

Table 13. Comparison of species groupings found in the PCA of the present study with groupings found in previous studies.

Cape Hatteras to Cape Chidley (present study)	Middle Atlantic Bight (Colvocoresses and Musick 1984)	Middle Atlantic Bight/Georges Bank/Gulf of Maine (Gabriel 1992)	Scotian Shelf (Mahon and Smith 1989*a*)	Scotian Shelf (Mahon 1997)
Gulf Stream flounder **Fourspot flounder** **Fawn cusk-eel** **Spotted hake** **Butterfish** Red hake Goosefish (angler)	Red hake Silver hake Gulf Stream flounder Spotted hake Butterfish Fourspot flounder	Spotted hake Fourspot flounder Butterfish	—	—
Blue hake **Rock grenadier** **Longnose eel** **Large-scale tapirfish** **Roughhead grenadier** Marlin-spike	—	—	—	—
Scup **Summer flounder** **Northern searobin** **Black sea bass** **Smooth dogfish**	Scup Summer flounder Northern searobin Black sea bass Smooth dogfish	Scup Summer flounder Northern searobin Black sea bass	—	—
Silver hake **Red hake** **Cusk** **Pollock** **Spiny dogfish** White hake Ocean pout Goosefish (angler) Haddock Shortfin squid		Red hake Silver hake Goosefish (angler) Cusk Spiny dogfish Shortfin squid	Red hake Goosefish (angler)	Red hake Silver hake Goosefish (angler) Spiny dogfish Spiny dogfish Shortfin squid

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Table 13 (*continued*).

Cape Hatteras to Cape Chidley (present study)	Middle Atlantic Bight (Colvocoresses and Musick 1984)	Middle Atlantic Bight/Georges Bank/Gulf of Maine (Gabriel 1992)	Scotian Shelf (Mahon and Smith 1989*a*)	Scotian Shelf (Mahon 1997)
Longhorn sculpin **Sea raven** **Yellowtail flounder** **Winter flounder** Ocean pout	Longhorn sculpin Yellowtail flounder American plaice Little skate Windowpane Spiny dogfish	Sea raven Ocean pout	Longhorn sculpin Sea raven Winter flounder Yellowtail flounder Winter skate	Longhorn sculpin Sea raven Winter flounder Yellowtail flounder Winter skate Ocean pout
Marlin-spike **Redfishes** **Witch flounder** **Black dogfish** Atlantic argentine White hake Longfin hake	Witch flounder White hake Goosefish (angler)	Redfishes Witch flounder White hake	Marlin-spike Longfin hake	Marlin-spike Redfishes Witch flounder White hake
Windowpane **Winter skate** **Little skate** Northern sand lance		Winter skate Windowpane Little skate		
Spotted wolffish **Atlantic wolffish** **Northern wolffish** Roughhead grenadier Atlantic cod Greenland halibut Redfishes	—			
Blackbelly rosefish **Offshore hake** **Shortnose greeneye**	Blackbelly rosefish Offshore hake Shortnose greeneye	Blackbelly rosefish Offshore hake Longfin hake Gulf Stream flounder	—	
Thorny skate **American plaice** Witch flounder Atlantic cod Smooth skate		Thorny skate American plaice	Thorny skate American plaice Atlantic cod	Thorny skate American plaice
Arctic cod **Atlantic sea poacher** Greenland halibut Polar sculpin	—	—	—	—
Fourbeard rockling **Atlantic hagfish** Smooth skate			Pollock Spiny dogfish	Cusk Pollock Atlantic argentine
Moustache sculpin **Alligatorfish** **Snowflake hookear sculpin**	—		Moustache sculpin	
Roughnose grenadier **Longfin hake** **Black dogfish**	—		Longfin hake	
Atlantic halibut **Haddock**	—	Atlantic halibut Atlantic wolffish	Atlantic halibut Haddock	Atlantic halibut Haddock Atlantic wolffish
Polar sculpin Roughhead grenadier Greenland halibut	—	—	—	—

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Table 13 (*concluded*).

Cape Hatteras to Cape Chidley (present study)	Middle Atlantic Bight (Colvocoresses and Musick 1984)	Middle Atlantic Bight/Georges Bank/Gulf of Maine (Gabriel 1992)	Scotian Shelf (Mahon and Smith 1989*a*)	Scotian Shelf (Mahon 1997)
Lumpfish	—	Lumpfish	—	—
Shorthorn sculpin				
Sea raven				
Cunner	—	Cunner	—	—
Winter flounder				
Snake blenny				

Note: Species groups in previous studies have been placed next to the PC assemblage from this study to which they appear most similar. In the present study column, the species in bold type have loadings >0.5; the species in standard type have loadings between 0.3 and 0.5. A dash indicates that none of the species in the present study's group were included in the previous studies.

projects, the East Coast of North America Strategic Assessment Project (ECNASAP) and the Cod Mortality Project, which share a common goal: description of coastal ecosystems and changes in their characteristics over time. ECNASAP is a collaboration between the NOAA/SEA and several Canadian agencies, including Environment Canada and the Department of Fisheries and Oceans. It has benefited from a synergistic development of new ideas and knowledge and from the efficiency of cooperative operations. We thank all those who have played a role in ECNASAP. This study comprises ECNASAP's Offshore Case Study which began in January 1995. The Department of Fisheries and Oceans' Cod Mortality Project started in 1995 and was planned to last 3 years. We thank Dan Basta and Doug Swain for valuable discussion and advice during the development of the study and Jack Musick for his comments on the manuscript.

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A mtDNA analysis of spatiotemporal distribution of two sympatric larval populations of rainbow smelt (*Osmerus mordax*) in the St. Lawrence River estuary, Quebec, Canada

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Abstract: Ecological isolation through resource partitioning is invoked as a major factor for explaining the persistence of genetically distinct yet closely related sympatric populations. Two genetically distinct sympatric populations of anadromous rainbow smelt (*Osmerus mordax*) exist in the middle estuary of the St. Lawrence River. The persistence of these coexisting populations in sympatry is in conflict with current theoretical concepts predicting population richness. In the present study, we performed mtDNA PCR–RFLP analysis of 922 larvae from 33 sampling stations to test the hypothesis that the larvae belonging to the two sympatric smelt populations of the St. Lawrence middle estuary are spatially segregated and that such segregation may promote the persistence of the populations. Results clearly revealed spatial homogeneity in the relative distribution of larvae from the two populations. Consequently, they did not support our working hypothesis that larvae belonging to the two sympatric smelt populations are spatially segregated. Two alternative explanations may account for the lack of spatial partitioning observed here. Competition may not be important enough to promote resource partitioning at the larval stage. Alternatively, resource partitioning occurs, but not spatially. This study also demonstrated that the effect of historical events may have been as important as contemporary ecological settings in determining genetic population structure in smelt.

Résumé : Plusieurs auteurs suggèrent que le partage des ressources écologiques est le principal facteur permettant le maintien de populations en sympatrie. Des travaux récents ont démontré l'existence de deux populations sympatriques d'éperlans arc-en-ciel (*Osmerus mordax*) anadromes génétiquement distinctes mais ayant le potentiel de s'hybrider dans l'estuaire moyen du St-Laurent. La persistance de populations en sympatrie dans ce milieu est contraire aux prédictions théoriques de l'hypothèse du « member–vagrant ». Dans cette étude, nous avons effectué une analyse de PCR–RFLP sur l'ADNmt de 922 larves d'éperlans représentant 33 sites d'échantillonnage dans le but de tester l'hypothèse voulant que ces deux populations larvaires soient spatio-temporellement ségrégées et que cette ségrégation explique en partie leur persistance dans le milieu. Les résultats de ces analyses démontrent une homogénéité spatiale dans la distribution des larves appartenant aux deux populations. Conséquemment, ces résultats ne supportent pas l'hypothèse de départ. On peut expliquer ces résultats de deux façons différentes. Premièrement, le partage des ressources est peu important au niveau larvaire pour l'éperlan du St-Laurent. Alternativement, le partage des ressources est significatif, mais ne se fait pas spatialement. Ces résultats démontrent de plus que les effets des événements historiques sur la structure des populations peuvent, dans certaines circonstances, être plus importants que les événements écologiques contemporains.

Introduction

Many north-temperate fish species are characterized by the occurrence of sympatric ecotypes that remain genetically distinct despite their potential to hybridize (summarized in Bernatchez et al. 1996). Because of the close relationships and incomplete reproductive isolation, these sympatric ecotypes have been studied to better understand the early stages of speciation. Ecological resource partitioning may contribute to decreasing the intraspecific competition between sympatric populations and thus favours the coexistence of populations by disruptive or frequency-dependent selection (e.g., Schluter and McPhail 1993; Robinson and Wilson 1994; Skúlason and Smith 1995).

Resource partitioning generally occurs in sympatric freshwater fish populations by trophic-based adaptations that are manifested by morphological, behavioural, and life history characteristics (e.g., Bernatchez and Dodson 1990; Taylor and Bentzen 1993*a*, 1993*b*; McPhail 1994; Skúlason and Smith 1995). Despite intensive studies of sympatric populations found in freshwater, no study has yet documented resource partitioning between sympatric fish populations of the same species in estuarine or marine ecosystems. Resource partitioning studies in marine ecosystems have only involved sympatric and reproductively isolated fishes (e.g., Schmitt and Holbrook 1986) but never sympatric populations with the possibility of interbreeding. Furthermore, all studies related to resource partitioning among sympatric fish populations have generally focused on juvenile and adult life history stages, but have not considered the larval stage despite the presumed importance

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of this stage in contributing to population richness and abundance (Sinclair 1988).

The rainbow smelt (*Osmerus mordax* Mitchill) is an osmerid species found in salinity ranging from freshwater to 30‰ and in water temperatures from below 0°C to more than 20°C (Nellbring 1989). It also exhibits a broad life history diversity consisting of anadromous and landlocked populations as well as freshwater dwarf and normal ecotypes (Nellbring 1989; Taylor and Bentzen 1993*a*) respectively specialized for planktivorous and (or) macrophagous and (or) piscivorous modes of life (Taylor and Bentzen 1993*a*, 1993*b*). Anadromous populations of this species exhibit a similar life cycle: adult smelt ascend streams and rivers in early spring and spawn in freshwater. Hatching occurs 1–2 weeks later and larvae from different spawning sites are usually transported within short periods into estuarine, fjord, or marine coastal waters where they are retained (Ouellet and Dodson 1985; Laprise and Dodson 1989*a*, 1989*b*) until metamorphosis. In the St. Lawrence estuary, the larvae are transported from spawning sites to a nursery zone situated in the maximum-turbidity zone where they are retained by active vertical migration (Dodson et al. 1989; Laprise and Dodson 1989*a*, 1989*b*).

Recent molecular studies demonstrated the existence of two genetically distinct ($F_{st} = 0.178$) sympatric populations in the middle estuary of the St. Lawrence River associated with two phylogenetically distinct lineages separated by about 0.8% average pairwise sequence divergence (Baby et al. 1991; Bernatchez and Martin 1996). Smelt ascending tributaries along the south shore of the estuary to spawn are dominated by phylogenetic group A (80.9%) whereas those caught in the north channel between Beauport and the Saguenay fjord are dominated by phylogenetic group B (87.1%) (Bernatchez and Martin 1996). Hereafter, these two populations are referred to as the "south shore population" and the "north shore population." Bernatchez (1997) demonstrated that the allopatric origin of phylogenetic groups A and B was associated with distinct glacial refugia and that the middle estuary represents a zone of secondary contact between these races that have developed biological barriers to gene flow between them. The diversification of these two populations does not appear to involve the same mechanisms that have been observed in the lacustrine environment. No evidence of dwarf and normal ecotypes has been documented, and the geographical mixing of the adults belonging to the two populations has been demonstrated outside the spawning locations (Bernatchez et al. 1995). These observations suggest that the coexistence of the sympatric populations may be determined by differential resource partitioning at a younger life history stage, namely at the larval stage. Coexistence of two sympatric larval populations within the middle estuary also apparently contradicts theoretical predictions on the ecological causes of population structure in the marine environment. According to the member–vagrant hypothesis (Sinclair 1988), population richness and structure are a function of the number and locations of geographic settings permitting the retention of early life history stages. The well-mixed upstream part of the St. Lawrence middle estuary associated with the smelt nursery area represents a unique oceanographic system for larval retention (Dodson et al. 1989). Consequently, only one population should be found in the system according to the member–vagrant model. The presence of the two populations in one oceanographic entity suggests that the larvae might use this structure differently. It is plausible that behavioural mechanisms evolved differently for each population resulting in distinct spatiotemporal distributions permitting coexistence in the same estuarine system (Laprise and Dodson 1989*a*, 1989*b*).

In this study, we performed a mtDNA polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) analysis to test the hypothesis that larvae belonging to the two sympatric smelt populations of the St. Lawrence middle estuary are spatially segregated and that such segregation may promote the coexistence of two populations in the system. As such, this study represents the first characterization of resource partitioning at the larval stage among sympatric fish populations and the first ecological assessment of sympatric fish populations in an estuarine environment.

Material and methods

Sample site

The St. Lawrence middle estuary is about 180 km long, receives an annual mean discharge of 10 000 m^3/s from the river, and comprises three main channels, north, middle, and south (e.g., Laprise and Dodson 1989*b*, 1993). The estuary is characterized by a well-mixed zone in the upstream section and a partially stratified zone downstream (El Sabh 1988). The salinity varies from 0‰ upstream of Île d'Orléans to over 30‰ downstream of Île aux Coudres (Fig 1). The estuary is highly energetic; tides vary between 3 and 5 m in height and current speed may reach 250 cm/s. Due to Coriolis force, tides, and the bottom topography of the estuary, important transverse currents are formed upstream and downstream of the central islands (Laprise and Dodson 1989*b*). Thus, this part of the St. Lawrence estuary between Île d'Orléans and Île aux Coudres is characterized by a cyclonic circulation that, in addition to the presence of a null zone (Dodson et al. 1989), contributes to the retention of suspended particulate matter inflowing from the river and salt marshes along the estuary forming a maximum-turbidity zone (Laprise and Dodson 1989*b*) (Fig. 1).

Sample collection

Larvae were sampled in the north and south channels over three consecutive months (Table 1). Pelagic trawling was carried out at five stations in May and six in June and July between Cap-Brulé and St-Siméon in the north channel and between St-Jean-Port-Joli and Cacouna in the south channel (Fig. 1). The stations were identified by the salinity of surface water masses. Larvae were also sampled twice in May at four stations around Île d'Orléans (Table 1; Fig. 1). Sampling consisted of step-oblique tows from the bottom to the surface using a 1-m plankton net equipped with a flowmeter and fitted with a 0.500-mm-mesh net for the sampling in May and around Île d'Orléans. Sampling in June and July was done in the same manner but with a 50-cm, 0.500-mm-mesh plankton net fitted to a Tucker trawl. The volume of water filtered was calculated from the distance towed and diameter of the net. Samples were preserved in 95% ethanol until genetic analysis.

Genetic analysis

Total DNA was extracted differently according to larval size. Small animals (ranging from 40 to 150 µg and from 5 to 13 mm in length) were soaked twice in distilled water for 15 min to remove ethanol. Water was discarded and each larva was put into 50 µL of extraction buffer (10 mM Tris–HCl (pH 8.0), 50 mM KCl, 0.5% Tween 20, 250 mg proteinase K/mL). Tubes were incubated for 3–4 h at 65°C and then heated at 95°C for 15 min. Digestion products were centrifuged for 10 min. For larger larvae, total DNA was purified

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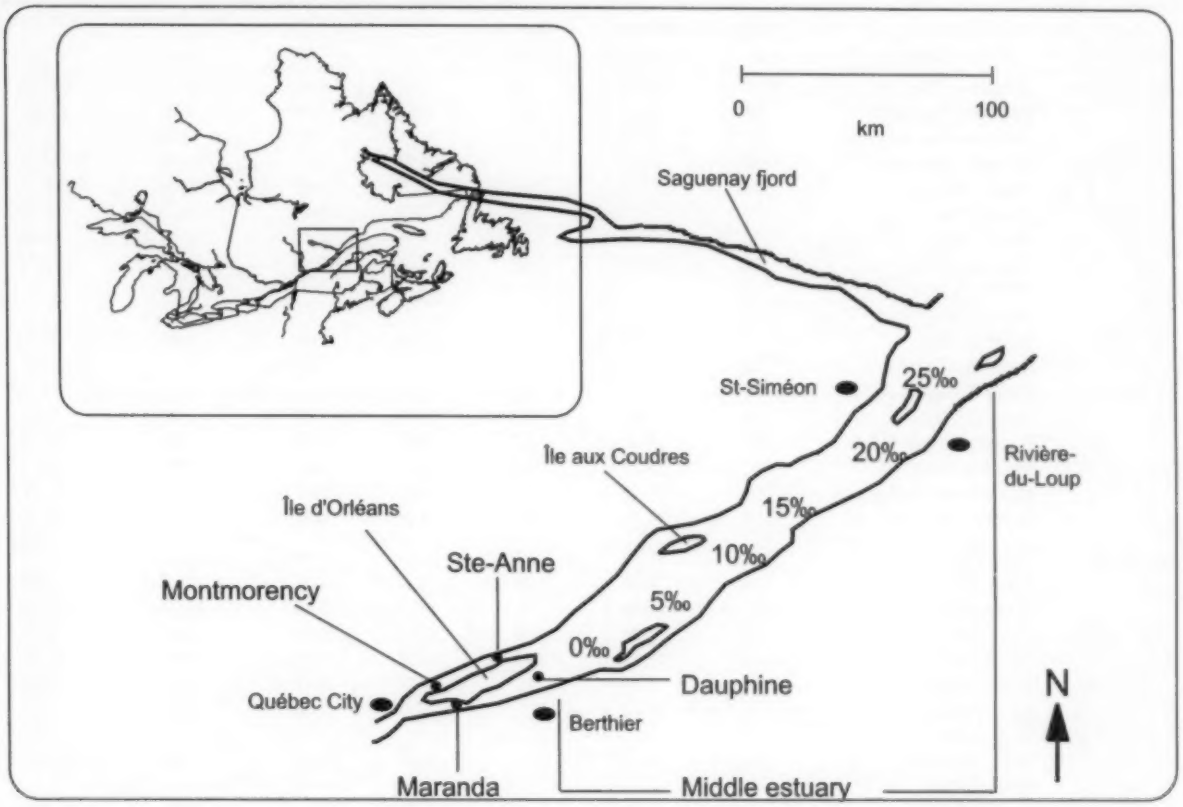
Fig. 1. Collection sites around Île d'Orléans and approximate locations of the six different water mass salinities (surface) that were sampled in the two channels of the middle estuary of the St. Lawrence River.



from the entire specimen following the procedures described in Bernatchez et al. (1992).

All larvae were allocated to mtDNA group A or B, defined previously by Bernatchez et al. (1995), by RFLP analysis performed with restriction endonucleases generating diagnostic sites on PCR-amplified segments. The analysis was initiated with the larger larvae. We performed PCR amplifications on a 2.4 kilobase pair (kb) segment encompassing the ND5 and ND6 subunits of NADH dehydrogenase using the primers developed by Cronin et al. (1993), which we named ND56R and ND56F. Each PCR reaction was composed of 1–5 µL of the total DNA extract (about 500 ng), 5 µL of 10× buffer (500 mM KCl, 100 mM Tris–HCl (pH9.0), 1% Triton X-100, 25 mM $MgCl_2$), 4 µL of dNTP mix (2.5 mM of each of dATP, dCTP, dGTP, and dTTP in sterile water), 2 µL of a 20 mM solution of the two primers, 2–4 units of *Taq* polymerase, and sterile water for a final volume of 50 µL per reaction. DNA amplification was performed in a programmable thermal cycler (Perkin-Elmer model 480) using the following profile: a preliminary denaturation at 95°C for 2 min followed by 40 cycles involving strand denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and primer extension at 72°C for 2 min and 30 s followed by a final elongation at 72°C for 10 min. The segments were subsequently digested with the hexameric enzyme *Apa* I used as recommended by the supplier (Pharmacia). This enzyme generated diagnostic fragment patterns for mtDNA groups A and B. mtDNA fragments were separated on 1.2 or 1.4% agarose gels at 90 V run for 5 h. Restriction fragments were revealed by ethidium bromide staining. Reliability was ensured by the analysis of 20 adult smelt characterized with RFLP analysis of the entire molecule and known to belong to mtDNA group A or B.

The small amount of DNA extracted from the small-size larvae in May was problematic for the amplification of the 2.4-kb segment. Development of primers for amplifying a shorter diagnostic segment was necessary to reduce the stringency of the PCR reactions. To do so, sequencing was performed on three potentially variable mitochondrial genes, the NADH subunits ND5 and ND6 and the ATPase subunit 6, to determine primer sets flanking diagnostic restriction sites between the two mtDNA groups (Fig. 2). PCR amplifications were performed on ND5 and ND6 subunit genes as described above and on the ATPase subunit 6 with the procedures described in Giuffra et al. (1994).

Amplified DNA was purified with the Quiagen DNA purification kit according to the supplier's recommendations. Double-stranded DNA sequencing was performed by applying the dideoxy chain-ending technique using the Sequenase kit (version 2.0, US Biochemical) as described in Bernatchez et al. (1992) with the following modifications. Sequence reactions were first performed with primers ND56R and ND56F for the ND5 and ND6 genes and H9208 and L8558 developed by Giuffra et al. (1994) for ATPase subunit 6. Internal primers developed in the present work were used for subsequent sequence reactions until the identification of diagnostic restriction sites between the two groups. Following this procedure, a 288 base pair (bp) segment of the ND6 gene localized between primers ND56R250 and ND56R450V was chosen to characterize the smallest larvae (Fig. 2). Diagnostic fragment patterns were generated

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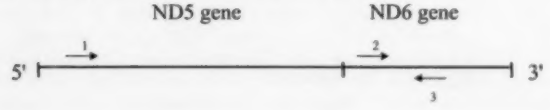
Table 1. Total number of caught larvae, density, samples sizes for genetic analysis, and relative frequency and absolute density of genetic groups A and B of rainbow smelt larvae for 42 stations in the St. Lawrence middle estuary for May 24–26, June 20–22, and July 15–16, 1994.

Month	Station	Total n	Density (n/100 m^3)	Genetic n	Relative frequency		Absolute density (n/100 m^3)	
					A	B	A	B
May 24–26	Dauphine (May 24)	2797	396.84	40	0.12	0.88	49.61	347.24
	Maranda (May 24)	135	20.11	43	0.05	0.95	1.01	19.10
	Montmorency (May 24)	210	36.85	24	0.00	1.00	0.00	36.85
	St-Anne (May 24)	130	32.63	37	0.03	0.97	0.88	31.75
	North (5‰)	766	179.20	40	0.05	0.95	8.96	170.24
	South (5‰)	50	9.22	36	0.03	0.97	0.28	8.94
	North (10‰)	75	9.60	33	0.15	0.85	1.44	8.16
	South (10‰)	396	65.18	40	0.05	0.95	3.26	61.92
	North (15‰)	0	—	—	—	—	—	—
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	3	0.43	2	0.00	1.00	0.00	0.86
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	0	—	—	—	—	—	—
	South (25‰)	14	1.83	10	0.00	1.00	0.00	1.83
	Dauphine (May 31)	237	35.46	38	0.05	0.95	1.88	33.58
	Maranda (May 31)	147	23.26	40	0.12	0.88	2.91	20.35
	Montmorency (May 31)	100	13.65	37	0.03	0.97	0.37	13.28
	St-Anne (May 31)	112	19.58	42	0.19	0.81	3.72	15.86
June 20–22	North (0‰)	154	11.46	41	0.10	0.90	1.12	10.34
	South (0‰)	6	0.49	5	0.2	0.8	0.10	0.39
	North (5‰)	1971	147.56	41	0.12	0.88	18.00	129.56
	South (5‰)	149	12.06	59	0.24	0.76	2.89	9.17
	North (10‰)	1172	77.79	43	0.12	0.88	9.02	68.77
	South (10‰)	15	1.18	12	0.17	0.83	0.20	0.98
	North (15‰)	16	1.12	7	0.00	1.00	0.00	1.12
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	24	1.62	14	0.00	1.00	0.00	1.62
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	5	0.42	5	0.00	1.00	0.00	0.42
	South (25‰)	241	17.29	21	0.00	1.00	0.00	17.29
July 15–16	North (0‰)	563	64.29	40	0.15	0.85	9.64	54.65
	South (0‰)	164	19.09	40	0.18	0.82	3.34	15.75
	North (5‰)	74	8.12	41	0.05	0.95	0.40	7.72
	South (5‰)	118	12.84	41	0.17	0.83	2.20	10.64
	South (10‰)	61	4.87	16	0.19	0.81	0.92	3.95
	North (15‰)	6	0.38	6	0.17	0.83	0.06	0.32
	South (15‰)	0	—	—	—	—	—	—
	North (20‰)	2	0.14	2	0.00	1.00	0.00	0.14
	South (20‰)	0	—	—	—	—	—	—
	North (25‰)	4	0.26	2	0.00	1.00	0.00	0.26
	South (25‰)	0	—	—	—	—	—	—

Note: Stations around Île d'Orléans are followed by the day of sampling and other stations are identified by channel and surface salinity.

Fig. 2. Primer locations on ND5 and ND6 genes in the rainbow smelt mtDNA molecule. ND56F300V (1) and ND56R450V (2) are located in the light strand, and ND56R250 (3) is located in the heavy strand. Primer 5′–3′ sequences are CCCGTTGCCCTTTACGTCACC for ND56F300V, GGACTACAAACAAAGTCAATAAG for ND56R450V, and ACTGGTCGTGTTTGTATAC for ND56R250.



by *Dde* I on this segment. Procedures to genetically characterize larvae with this segment were done as described for the 2.4-kb segment, except that the elongation step was reduced to 45 s.

Data analysis

The density of larvae at each station (number of larvae per 100 m^3 of water filtered) was calculated at each station for each month and averaged. These estimates allowed us to quantitatively evaluate the number of larvae belonging to each genetic group, and therefore, each population, for the different stations or periods of sampling.

Heterogeneity in the distribution of larval populations among stations, groups of stations, and sampling periods was first evaluated by the analysis of frequency distribution of the two genetic groups for

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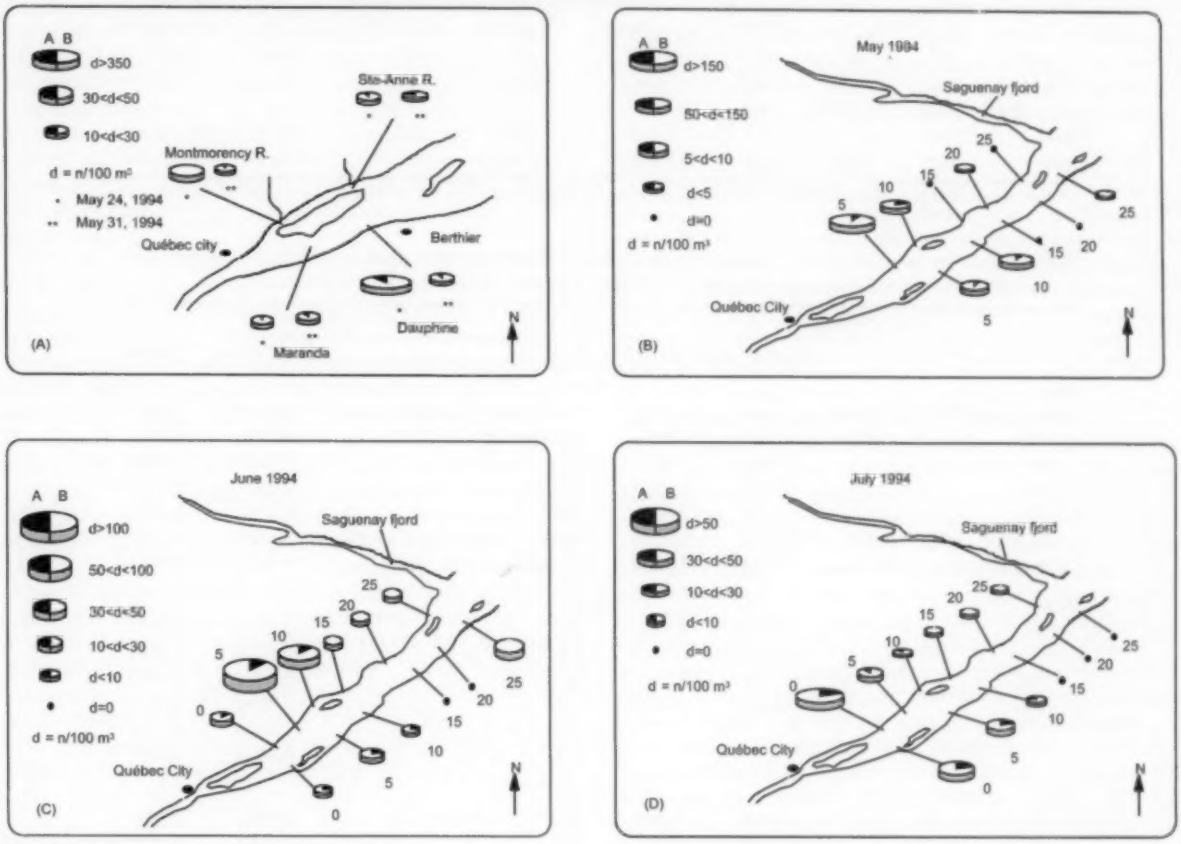
Fig. 3. Frequency distribution and abundance (d = number of larvae/100 m^3 of filtered water) of larvae belonging to mtDNA groups A and B for (A) around Île d'Orléans on May 24 and 31, 1994, and (B–D) in the middle estuary for the two channels in May, June, and July, respectively. Names or numbers above pie charts identify sampling stations.



each station or group of stations (in cases where no distinction was detected among stations) using chi-square randomization tests (Roff and Bentzen 1989) with 1000 randomizations performed by the MONTE program of the REAP software package (McElroy et al. 1992). Significant heterogeneity was accepted when the probability (p) was <50/1000.

The relative contribution of the two breeding populations to larval samples was estimated for each station and group of stations for each period of sampling. This was calculated using the method of Lane et al. (1990) by first considering the frequency of genetic groups A and B in the two reproductive stocks characterized previously as baseline populations (Bernatchez et al. 1995). Final estimates, however, were calculated by using the distribution of the two mtDNA groups in May larvae as the baseline data for the north shore population. This substitution generated more plausible estimates (see Results section).

Results

Sequence analysis

Sequence analysis was performed until suitable primers flanking diagnostic restriction sites were identified. This resulted in a 5′–3′ sequence effort of 3042 bp of the light strand of mtDNA (Gene Bank accession Nos. AF034748–AF034752). Sequences were obtained for one representative of each mtDNA group: (*i*) 618 bp from the 68th nucleotide of the ATPase subunit 6 to 10 bp in the adjacent cytochrome oxidase subunit 3 (COIII) gene, (*ii*) the complete ND5 gene (1833 bp) and 32 bp of the adjacent leucine tRNA gene, and (*iii*) the complete ND6 gene (522 bp) and 28 bp of the adjacent glucine tRNA gene. Nine mutations were observed in these sequences and their spatial distribution was heterogeneous. The ATPase subunit 6 gene represented the least variable region, with no substitutions detected, compared with six in the ND5 gene and three in the ND6 gene discriminating the two mtDNA groups. The overall sequence divergence estimate between the groups was 0.30%. Seventy-eight percent (seven of nine) of these substitutions were transitions, three involving purine–purine differences (all in the ND5 gene) and four involving pyrimidine–pyrimidine differences (one in the ND5 gene and three in the ND6 gene).

Larval abundance and distribution

Zero to 2797 larvae were captured at each of the 42 stations in May, June, and July 1994 (Table 1; Fig. 3). Low densities of larvae were observed at the downstream sites of the middle estuary. Thus, at the 18 stations having a salinity between 15 and 25‰ and for the three sampling months, 17 had a larval

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Table 2. Total number of larvae belonging to mtDNA groups A and B, their ratio, south shore contribution and variance estimates, abundance of larvae in the south and north shore populations in the St. Lawrence middle estuary, and their ratio for each period of sampling.

	Total *n*		A:B	Contribution	Variance	Abundance (*n*/100 m^3)		South:north
	A	B	ratio	(%)	(%)	South	North	ratio
May 24	65.44	686.89	1:10.5	0	0.086	0	730.2	—
May 31	8.88	83.07	1:9.4	—	—	—	—	—
June	31.33	222.37	1:7.1	5.0	0.109	12.7	241.0	1:19.0
July	16.92	95.22	1:5.6	8.7	0.173	9.7	102.4	1:10.6

Note: No contribution and variance estimates were calculated for May 31, since only Île d'Orléans was sampled on this date.

Table 3. Relative contribution and variance estimates of the south shore rainbow smelt population (dominated by genetic group A) to the larval samples around Île d'Orléans and from the St. Lawrence middle estuary.

South channel			North channel		
	Contribution	Variance		Contribution	Variance
May			May		
Dauphine (May 24, 1994)	0.0512	0.0033	Montmorency (May 24, 1994)	0.0000	0.0010
Maranda (May 24, 1994)	0.0000	0.0019	St-Anne (May 24, 1994)	0.0000	0.0016
5‰	0.0000	0.0017	5‰	0.0000	0.0020
10‰	0.0000	0.0020	10‰	0.0873	0.0043
25‰	0.0000	0.0010	20‰	0.0000	0.0010
Dauphine (May 31, 1994)	0.0000	0.0021	Montmorency (May 31, 1994)	0.0000	0.0016
Maranda (May 31, 1994)	0.0526	0.0033	St-Anne (May 31, 1994)	0.1427	0.0041
June			June		
0‰	0.1565	0.0313	0‰	0.0145	0.0028
5‰	0.2119	0.0035	5‰	0.0485	0.0032
10‰	0.1108	0.0117	10‰	0.0401	0.0030
25‰	0.0000	0.0010	15‰	0.0000	0.0010
			20‰	0.0000	0.0010
			25‰	0.0000	0.0010
July			July		
0‰	0.1219	0.0041	0‰	0.0873	0.0037
5‰	0.1163	0.0039	5‰	0.0000	0.0019
10‰	0.1399	0.0097	10‰	0.1108	0.0062
			15‰	0.1108	0.0228
			20‰	0.0000	0.0010
			25‰	0.0000	0.0010

Note: Date of sampling given in parentheses.

density <2/100 m^3 water (Table 1). Larval density was much higher in the upstream section of the middle estuary (≤ 10‰), with the maximum reached at pointe Dauphine in May where a density of 396.8 larvae/100 m^3 was observed.

Two to 59 larvae per station were genetically characterized (all larvae when $n < 40$ and random subsampling of 40–59 for the others) for a total of 922 larvae from 33 stations. No larvae were caught at the other stations. The frequency distribution of the larvae belonging to mtDNA groups A and B did not support the hypothesis of differential spatial segregation between the two larval populations. Thus, the distribution of larvae characterized by either mtDNA group A or B was very homogeneous among stations (Table 1). Larvae of mtDNA group B dominated each station at a frequency varying between 76 and 100% (Table 1; Fig. 3), and no difference in the frequency distribution of the two mtDNA groups was detected among individual samples for each month. Heterogeneity analysis was then performed each month among stations of the north and south channels individually, and no significant difference was observed except at Montmorency River and St-Anne River on May 31. Samples of each channel were then pooled for each month to compare the distribution of mtDNA groups between channels (May 31 observations excluded) and no heterogeneity was detected. Finally, samples were pooled by month to test for temporal heterogeneity of frequency distribution which indicated distinct distributions of the two mtDNA groups between May and June and May and July. The differences were explained by an increase in the number of larvae belonging to group A over time. This increase is illustrated by the ratio of absolute numbers of larvae characterized by mtDNA groups A and B for a given month (Table 2).

The distribution of mtDNA groups A and B among samples allowed the estimation of the relative contribution of the south shore breeding population to the number of larvae in each

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station and for each month (Table 3). Initially calculated using the baseline populations of Bernatchez et al. (1995), contribution estimates of the south shore population were negative for May. Such results were due to the frequency of mtDNA group A (6%) observed in May, which was lower than the frequency reported by Bernatchez et al. (1995) for the north shore breeding population (13%). As the sample size for May 24–26 sampling was greater (n = 305 compared with 101) than that of Bernatchez et al. (1995), and always avoided negative contributions, we felt that the proportion of larvae belonging to groups A and B in this month was more representative of the north shore baseline population for estimating relative contributions.

The estimated contribution of the south shore population to larval samples was very small and homogeneous among stations for a given month (Table 3). Seventeen of the 33 contribution estimates were null and the average contribution of the 16 others was 10.0%. A temporal increase in the frequency of mtDNA group A was also reflected by the estimate of the south shore contribution to larval samples (pooled) for each month. Thus, the estimated contribution for this population increased from 0% in May to 5% in June and to 8.7% in July (Table 2).

The total larval density belonging to the south shore population in the system was calculated each month by multiplying the contribution estimate of the south shore population by the larval density for each month. The total abundance estimates of larvae belonging to the north shore population were estimated by the subtraction of the south shore larval density from the total larval density. No detectable contribution of the south shore population was observed in May. For the other two months, the larval south to north shore population density ratios were about 1:20 in June and 1:10 in July (Table 2). Therefore, the total larval density indicated an increase of the larvae belonging to the south shore population over time.

Discussion

Sequence analysis

Sequence analysis revealed heterogeneity in the mutational distribution between the two smelt mtDNA groups, suggesting differential mutation rates among genes, which corroborate previous studies (e.g., Meyer 1994; Zardoya et al. 1995). The divergence estimates between the two mtDNA groups were 0.0% (0/638) for ATPase subunit 6, 0.33% (6/1830) for the ND5 gene, and 0.57% (3/522) for the ND6 gene. However, an unexpected result was the overall low divergence (0.29%) between the two mtDNA groups obtained by sequencing compared with RFLP analysis performed over the entire mtDNA molecule (about 0.8%) (e.g., Bernatchez and Martin 1996). It has generally been reported that RFLP analysis detects less variation than sequencing because it is an indirect method of variation detection (e.g., Meyer 1994). The almost threefold difference in divergence estimates between the two methods indicates that the regions chosen for sequencing were less variable than averaged over the entire molecule. This was unexpected, since the chosen mtDNA regions were reported to be highly variable at the intraspecific level in other fishes, namely brown trout (*Salmo trutta*) (Giuffra et al. 1994; Bernatchez and Osinov 1995) and lake whitefish (*Coregonus clupeaformis*) (Pigeon et al. 1997). These results also suggested that it may not be possible to generally classify mtDNA genes as highly variable or not, since the extent of polymorphism in specific genes is apparently species dependent as stated above (also see Taylor and Dodson 1994). As mutation rate in mitochondrial protein-coding genes is mainly affected by selective functional constraints on the gene product (Meyer 1994), these results suggest that different mtDNA genes may be subject to differential selective constraints even among closely related species.

Analysis of smelt larvae

This study revealed four relevant results to the issue of the sympatric persistence of fish populations and to the biology of smelt in the estuary: the overall similarity of larval density distribution with previous studies, the lack of spatial segregation over time between the two larval populations, the increase of the south shore larvae relative to the north shore larvae with time and finally, the overall greater abundance of the north shore larval population.

Temporal stability of larval density distribution

Our results indicated the upstream displacement of larvae during the season, as maximum densities moved from water masses between 5 and 10‰ in May to waters of less than 5‰ in July. These results are identical to those obtained in detailed studies of smelt larval ecology in the St. Lawrence estuary. The low density of larvae in the downstream section of the middle estuary compared with the upstream section was previously described by Laprise and Dodson (1989*a*, 1989*b*). Larval dispersal seemed also to follow the same pattern described previously (e.g., Laprise and Dodson 1989*a*). Briefly, early after hatching, the larvae are transported into the estuarine nursery zone where they are retained by active vertical displacements in combination with the circulation system. The fact that older larvae use the tidal currents more efficiently by increasing the amplitude of their vertical migration explains the increase in density farther upstream in July (Laprise and Dodson 1989*b*). These results indicate that the adaptive mechanisms of vertical displacements exhibited by smelt larvae for their retention in a highly productive nursery area (Dauvin and Dodson 1990) are temporally stable.

Resource partitioning

Our results revealed spatial homogeneity in the relative distribution of the larvae from the south and north shore populations. Consequently, they did not support our working hypothesis that the larvae belonging to the two sympatric smelt populations in the St. Lawrence middle estuary are spatially segregated and that such segregation may promote in part the persistence of two populations in the system. Two alternative explanations may account for these observations.

First, the persistence of the two larval populations without apparent segregation may indicate that there is not sufficient competition to drive resource partitioning in the system at the larval stage. The maximum-turbidity zone of St. Lawrence middle estuary where the maximum density of smelt larvae is found represents a productive food web with high densities of both micro- and macro-zooplankton (e.g., Dodson et al. 1989). The densities of potential food for larvae, including copepods and *Neomysis americana*, are 10 and 100 times greater, respectively, within the turbidity zone than at its downstream limit (Dodson et al. 1989; Dauvin and Dodson 1990). This

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abundance is presumably due to the common hydrodynamic control of suspended sediment and some pelagic components of the estuarine ecosystem (e.g., Laprise and Dodson 1989*a*, 1989*b*). The high content of food resources in this zone is also illustrated by a growth rate of smelt larvae of 0.33 mm/day within the turbidity zone as compared with growth rates varying from 0.10 to 0.24 mm/day downstream of the turbidity zone (Laprise and Dodson 1989*b*). In addition to the abundance of food resources, the existence of only one potential interspecific competitor, the Atlantic tomcod (*Microgadus tomcod*), renders plausible the persistence of coexisting smelt populations without spatial resource partitioning at the early larval stages. However, as suggested by the development of spatial segregation between tomcod and smelt later in the season (Laprise and Dodson 1989*b*), spatial segregation between the two smelt populations may also occur later in life in relation to an unknown resource gradient.

The second alternative explanation for these observations may be related to a temporal delay in the prey consumed by larvae of each population. The present results revealed a very low abundance, and possibly an absence, of larvae belonging to the south shore population in May and their gradual increase over time. This is indicative of a temporal delay in the entrance of the two larval populations to the estuary. These observations corroborate present knowledge of the breeding ecology of smelt. Briefly, although the north shore spawning locations are unknown, the capture of spent adults in early spring indicated that the breeding period takes place at the latest around the end of April (Bernatchez et al. 1995), possibly directly in the St. Lawrence River. In contrast, the south shore population breeding period has been well defined to occur 2–4 weeks later in the south shore tributaries (Ouellet and Dodson 1985). Assuming similar developmental rates, these observations imply that larvae of the south shore population are smaller than those of the north shore population at any given time. Many studies have demonstrated that the prey eaten varies with the length of the smelt larvae (e.g., Nellbring 1989; Dauvin and Dodson 1990). For the St. Lawrence middle estuary, Dauvin and Dodson (1990) observed that calanoid copepods were the major prey item for all length-classes, but mysids were proportionately more abundant among large larvae (>30 mm) and cladocerans were proportionately more abundant in the intermediate class (25–30 mm). These prey preferences may contribute to reducing diet overlap among larvae of different sizes. Globally, these observations are congruent with a possible resource partitioning by time lag between breeding periods of the two populations in the middle estuary. This hypothesis, however, remains to be tested by more detailed studies on the trophic ecology of smelt larvae belonging to each population.

Member–vagrant hypothesis versus historical factors

Whatever the explanation for the lack of spatial segregation of smelt larval populations in the middle estuary, their sympatric occurrence is in conflict with current theories predicting population richness in aquatic environments. Namely, the member–vagrant hypothesis (Sinclair 1988) predicts that the number of populations composing a given species will primarily be determined by the number of environmental settings (geographic, physical, oceanographic) to which (*i*) spawning adults are adapted to home and (*ii*) early life history is behaviourally adapted to permit retention. This implies that in situations where the distribution of the larvae is different from the distribution of the spawning sites, such as is the case with smelt in the middle estuary, the population structure should reflect the number of retention zones rather than the number of spawning sites (Baby et al. 1991). The middle estuary is characterized by a well-mixed zone in the upstream part and a partially stratified zone in the downstream part (El Sabh 1988). The well-mixed upstream part of the St. Lawrence middle estuary associated with the smelt nursery represents a unique oceanographic system (Dodson et al. 1989) within which smelt larvae are maintained by active and passive processes (Laprise and Dodson 1989*a*, 1989*b*). Furthermore, the general cyclonic circulation of the estuary permits the formation of strong transversal currents (Dodson et al. 1989) that promote the transport and mixing of the larvae of both populations across the estuary. As the mixing of the young life history stages reported in this study occurs in the middle estuary, the member–vagrant hypothesis would support the existence of a single population in that system.

The sympatric occurrence of both larval populations in a single retention zone therefore indicates that contemporary ecological settings may not be the most important mechanisms driving population divergence. It has been demonstrated that the two smelt populations of the middle estuary are derived from distinct glacial races that developed partial reproductive barriers (Bernatchez and Martin 1996; Bernatchez 1997). The fact that gene flow between them has been less than between any other anadromous ones studied in northeastern North America (e.g., Taylor and Bentzen 1993*a*; Bernatchez and Martin 1996) indicates that genetic divergence due to long-term geographic isolation may have been more important than contemporary ecological conditions in determining their divergence and persistence. As such, our results reemphasize the importance of considering not only ecological processes but also the evolutionary history to better understand the mechanisms determining a species' population structure.

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DNA and allozyme markers provide concordant estimates of population differentiation: analyses of U.S. and Canadian populations of Yukon River fall-run chum salmon (*Oncorhynchus keta*)

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Abstract: Although the number of genetic markers available for fisheries research has steadily increased in recent years, there is limited information on their relative utility. In this study, we compared the preformance of different "classes" of genetic markers (mitochondrial DNA (mtDNA), nuclear DNA (nDNA), and allozymes) in terms of estimating levels and partitioning of genetic variation and of the relative accuracy and precision in estimating population allocations to mixed-stock fisheries. Individuals from eight populations of fall-run chum salmon (*Oncorhynchus keta*) from the Yukon River in Alaska and Canada were assayed at 25 loci. Significant differences in mitochondrial haplotype and nuclear allele frequencies were observed among five drainages. Populations from the U.S.–Canada border region were not clearly distinguishable based on multilocus allele frequencies. Although estimates of total genetic diversities were higher for the DNA loci ($H_t = 0.592$ and $h = 0.647$ for nDNA and mtDNA, respectively) compared with protein allozymes ($H_t = 0.250$), estimates of the extent of population differentiation were highly concordant across marker classes (mean $\theta = 0.010$, 0.011, and 0.016 for allozymes, nDNA, and mtDNA, respectively). Simulations of mixed-stock fisheries composed of varying contributions of U.S. and Canadian populations revealed a consistent bias for overallocation of Canadian stocks when expected Canadian contributions varied from 0 to 40%, due primarily to misallocations among genetically similar border populations. No single marker class is superior for differentiating populations of this species at the spatial scale examined.

Résumé : Ces dernières années, de nouveaux marqueurs génétiques sont régulièrement venus enrichir la panoplie d'outils dont dispose la recherche dans le domaine des pêches; toutefois, l'utilité relative de ces instruments reste en grande partie à déterminer. Nous avons comparé la performance de différentes « classes » de marqueurs génétiques (ADN mitochondrial (ADNmt), ADN nucléaire (nADN) et alloenzymes) d'après leurs propriétés de différenciation et le degré de variation génétique qu'ils permettent de mettre en évidence et d'après leur exactitude et leur précision relatives pour l'estimation de la distribution des populations dans les pêche de stocks mixtes. Nous avons étudié 25 locus chez des spécimens prélevés dans huit populations de saumon kéta (*Oncorhynchus keta*) à montaison automnale du fleuve Yukon, en Alaska et au Canada. L'analyse des haplotypes mitochondriaux et des fréquences alléliques nucléaires a permis de mettre en évidence des différences significatives entre cinq bassins hydrographiques. La mesure de la fréquence allélique de locus multiples n'a pas permis de mettre en évidence des différences nettes entre les populations de la région limitrophe canado-américaine. La diversité génétique totale mesurée d'après les locus d'ADN ($H_t = 0{,}592$ et $h = 0{,}647$, respectivement, pour l'ADNn et pour l'ADNmt) était plus grande que celle mise en évidence au moyen des alloenzymes ($H_t = 0{,}250$); par ailleurs, pour ce qui est de la différenciation des populations, tous les marqueurs concordaient fortement (la moyenne du facteur θ étant respectivement de 0,010, 0,011 et 0,016 pour les alloenzymes, l'ADNn et l'ADNmt). Dans des simulations reproduisant la pêche de stocks mixtes diversement composés de populations américaines et canadiennes, nous avons constaté un biais constant dénotant la sur-allocation des stocks canadiens lorsque la part des populations canadiennes prévue était comprise entre 0 et 40%, ce phénomène étant dû avant tout à des erreurs d'allocation concernant des populations limitrophes génétiquement ressemblantes. À l'échelle spatiale considérée, aucune des classes de marqueurs évaluées n'a surpassé les autres pour la différenciation des populations de saumon kéta.

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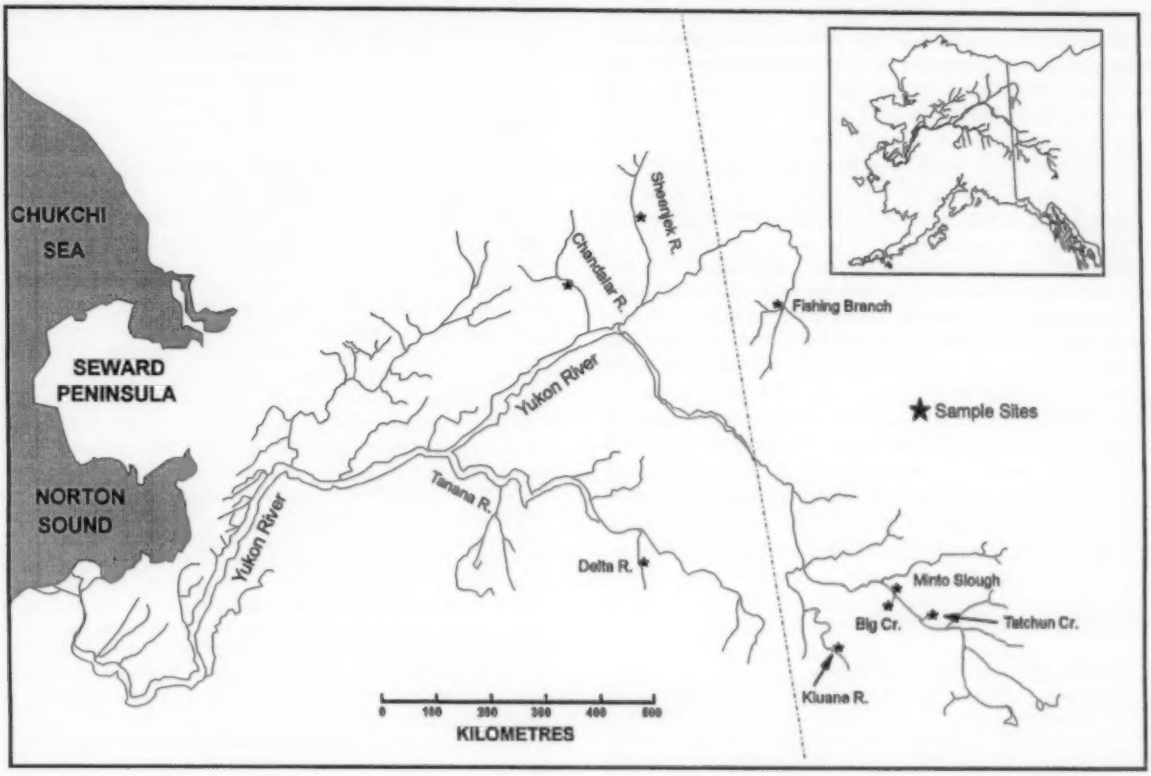
Fig. 1. Map of the Yukon River drainage showing sampling locations.



Introduction

Recent advances in molecular technology have dramatically increased the number of markers capable of resolving variation at the DNA level (see reviews by Park and Moran 1994; Ferguson et al. 1995). Different techniques including mitochondrial DNA (mtDNA) (Meyer 1993), minisatellites (O'Reilly and Wright 1995), and microsatellites (Scribner et al. 1996) have the ability to target DNA sequences that have different patterns of inheritance, different modes and rates of mutation, and, concomitantly, different levels of variation. It is not intuitively clear which genetic markers afford the greatest utility for elucidating population variation. Can DNA markers improve discrimination among salmon populations beyond the obvious advantage of providing more loci? Can the relative utility of different markers vary at different spatial scales of analysis? Unfortunately, little attempt has been made to survey the same set of populations for multiple classes of genetic markers or to assess the accuracy and precision of various genetic markers (or mixtures of different genetic loci analyzed using protein- and DNA-based methodologies) for mixed-stock analysis (MSA).

Chum salmon (*Oncorhynchus keta*) have received considerable attention due to their broad geographic distribution and abundance in U.S.–Canada border drainages. This important international fisheries resource can be used as a model system for comparative genetic studies. Many fisheries for this species are conducted in locations far removed from the breeding locations from which individuals have originated, and as such are typically composed of a mixture of populations from a variety of geographic locales.

The Yukon River drainage is the largest drainage used by chum salmon in the northern portion of their range and is one of two drainages in western Alaska that support both summer- and fall-run populations. Summer-run chum salmon spawn during July and early August in the lower and middle portions of the river (Buklis 1981), while fall-run fish generally spawn in September and October in the middle and upper portions of the river (Buklis 1981). Fall-run chum salmon are somewhat unique because while most chum salmon do not migrate great distances in freshwater, Yukon River fall-run chum salmon migrate nearly 3000 km to spawning areas in both Alaska and the Yukon Territory of Canada. This extensive geographic distribution within the drainage may promote a high degree of population genetic structuring, which in turn may be used in MSA.

Much of the current information on population genetic characteristics of chum salmon and of stock composition of chum salmon fisheries has come from allozyme loci (Phelps et al. 1994). These studies and references cited therein reveal that chum salmon vary in their genetic characteristics both spatially and temporally (e.g., based on run-timing). Surveys of mtDNA (Park et al. 1993) and minisatellite DNA variation (Beacham 1996) provide additional insights into the extent of population structuring at local and regional geographic scales.

Significant differences in allozyme allele frequencies were

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observed between fall- and summer-run populations in the Yukon River (Wilmot et al. 1994). Allozyme data could successfully be used to separate fall- and summer-run chum salmon when they occurred together in mixtures. However, allozyme data were not sufficient to separate fall-run chum salmon of U.S. versus Canadian origin due to genetic similarities among populations spawning in drainages near the U.S.–Canada border (Porcupine River and Yukon River mainstem; Fig. 1). Development and screening of additional genetic markers was deemed appropriate to increase statistical power (Wilmot et al. 1992).

The objectives of this study were to (*i*) describe the magnitude and patterns of population differences in allele and haplotype frequency among fall-run chum salmon within the Yukon River drainage using allozyme, nuclear DNA (nDNA), and mtDNA markers, (*ii*) examine the utility of various genetic markers for population separation and MSA and discuss options and criteria for choosing subsets of markers for particular descriptive and analytical purposes, (*iii*) compare equal and proportional weighting strategies for genetic MSA, and (*iv*) address issues related to definitions of reporting groups based on political (United States and Canada) and biological (i.e., tributary of origin or geographic proximity) criteria.

Methods and materials

Sampling locations and tissue collections

Samples were obtained from >50 adult fall-run chum salmon from each of eight populations (Fig. 1) within the tributaries or mainstem of the Yukon in the United States and Canada. Specific sampling locales and years of collection include three populations from the United States (Delta River (1990), Chandalar River (1989), and Sheenjek River (1989)) and five populations from Canada (Big Creek (1992), Minto Slough (1989), Tatchun Creek (1992), Fishing Branch Creek (1989), and Kluane River (1992)). Collections were made on the spawning grounds during September–November of each year. Although samples were not all obtained from the same year, previous studies (Wilmot et al. 1992; Kondzela et al. 1994) have shown that for natural populations, allozyme allele frequencies within a sampling locale are temporally stable (in the absence of hatchery influences, e.g., Phelps et al. 1994). We assumed that allele frequencies at the DNA loci surveyed in the present study were also temporally stable.

Four tissues (skeletal muscle, liver, eye, and heart) were taken from each individual and frozen immediately in individually labeled tubes. All samples were kept at –70°C in the laboratory until analyzed. DNA was extracted from muscle or heart for each individual using proteinase-K followed by standard phenol–chloroform extraction and alcohol precipitation (Sambrook et al. 1989). DNA concentrations were determined using florimetry, and stocks of 50 ng/μL were made for all individuals. DNA samples were kept at –20°C.

Allozymes

Protein electrophoresis followed standard techniques as described by Aebersold et al. (1987). Locus nomenclature followed recommendations adopted by the American Fisheries Society (Shaklee et al. 1990). Allelic designations were standardized following previous studies of chum salmon (e.g., Kondzela et al. 1994). Twenty loci (including two isoloci) which have previously been described as polymorphic for Yukon River chum salmon from these locales (Wilmot et al. 1992) were used for analysis. Loci include *sAAT-1,2**, *sAAT-3**, *mAAT-1**, *mAH-3**, *ALAT**, *ESTD**, *G3PDH-2**, *bGLUA**, *mIDHP-1**, *sIDHP-2**, *LDH-A1**, *sMDH-A1**, *sMDH-B1,2**, *mMEP-2**, *MPI**, *PEP-B1**, *PEPLT**, and *PGDH**.

Nuclear DNA loci

Five microsatellite loci were used for analysis (*One*μ1, *One*μ10, and *One*μ18, Scribner et al. 1996; *Ots*1, Banks et al. 1996; *Ssa*14, McConnell et al. 1995). Loci were screened for variation using polymerase chain reaction (PCR) conditions optimized for each locus. All PCR reactions were conducted in 25-μL volumes using about 100 ng of template DNA, 0.25 unit of Taq DNA polymerase (Perkin Elmer), and buffer (10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) for 35 cycles. Primer and dNTP concentrations were 1 pmol of each primer and 25 μM of each dNTP (*One*μ1, *One*μ10, and *One*μ18), 8.3 pmol of each primer and 40 μM of each dNTP (*Ots*1), and 10 pmol of each primer and 200 μM of each dNTP (*Ssa*14). Samples were screened for variation using [γ-^{32}P]ATP end-labeled primers. One primer from each pair was end-labeled using T4 polynucleotide kinase according to manufacturer's specifications (Pharmacia). PCR conditions were as described above using locus-specific annealing temperatures (*One*μ1, *One*μ10, and *One*μ18: 55°C; *Ots*1: 58°C; *Ssa*14: 50°C). Each PCR reaction was mixed with 10 μL of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, 0.05% bromphenol blue) and heated for 5 min at 95°C before loading onto a 6% denaturing sequencing gel. An M13 control sequencing reaction (USB) was run adjacent to the samples to provide an unambiguous size marker for the microsatellite alleles. Gels were dried and autoradiographed overnight at –70°C using intensifying screens.

Primers GH2ex4 and GH2ex5a described in Park et al. (1995) were used to amplify intron D of the growth hormone 2 gene (*GH2D**). PCR reactions were conducted in a total volume of 100 μL with a final concentration of 2 mM $MgCl_2$, 0.8 mM each dNTP, 0.2 mM of each primer, 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer), and 0.1–0.4 μM genomic DNA template. Cycling conditions included an initial denaturation at 94°C for 1 min followed by 40 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. A final extension was performed at 72°C for 5 min. Polymorphisms were detected by restricting the PCR product with *Hinf* I following manufacturer's recommendations (New England Biolabs). Digested products were run on a 4% Amplisize agarose gel (BioRad) at 50 V for 4.5 h. Resulting banding patterns were visualized using ethidium bromide under ultraviolet light.

mtDNA variation

PCR was used to amplify a portion of the ND5/6 region of the mitochondrial genome (about 2400 base pairs) using primers 5′-AATAGCTCATCCATTGGTCTTAGG-3′ and 5′-TAACAACGG-TGGTTTTTCAAGTCA-3′ reported in Cronin et al. (1993). PCR reactions were conducted in 50-μL volumes using 100–500 ng of DNA, 10× buffer (0.1 M Tris–HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl, 1 mg bovine serum albumin/mL), 200 mM of each dNTP, 10 pmol of each primer, and 1.25–2.5 units of Taq DNA polymerase (Perkin Elmer). Amplification consisted of one denaturation cycle of 95°C for 4 min followed by 32 cycles of 95°C for 50 s, 50°C for 40 s, and 70°C for 2.5 min.

PCR products were digested with seven restriction enzymes (*Alu* I, *Ase* I, *Bsa* JI, *Dpn* II, *Rsa* I, *Sau* 96I, and *Taq* I) under conditions specified by the manufacturer (New England Biolabs). Digested PCR products were run on 2% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light (312 nm). Restriction fragment sizes were estimated by comparison with a 100 base pair ladder (Pharmacia) and a phage lambda standard (Lambda c1857 Sam 7 digested with *Hind* III and PhiX174 digested with *Hae* III; Pharmacia). Composite haplotypes were defined based on presence/absence of restriction sites across all restriction enzymes (Lansman et al. 1981).

Statistical analyses

Allele and haplotype frequencies were calculated for each of the eight populations. Tests for departures from Hardy–Weinberg

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equilibrium were conducted for each nuclear locus ($\alpha = 0.05$, adjusted for multiple testing using the Bonferroni criterion, e.g., Manly 1985). Estimates of total gene diversity (Nei 1973) and the number of alleles were estimated for each nuclear locus across the eight populations. For microsatellite loci *Ots*1 and *Ssa*14, rare alleles of similar size were binned into composite allelic classes. All statistical analyses were based on composite allele frequencies.

Summarization with *F*-statistics (Cockerham 1969) the partitioning of allelic variance at each of the 24 nuclear loci among individuals within and among populations was conducted using the program FSTAT (Goudet 1995). Tests of significance were conducted using permutations ($N = 1000$) of alleles within samples (f) and of alleles or multilocus genotypes among samples (θ). In a complimentary analysis of spatial heterogeneity in mtDNA haplotype frequencies, the analysis of molecular variance (AMOVA) program (Excoffier et al. 1992) was used to estimate θ. To facilitate comparisons across the three markers classes (allozymes, nDNA, and mtDNA), the same genetic distance metric (Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967)) was used in metric multidimensional scaling (MDS) (Krzanowski and Marriott 1994) to describe genetic relationships among populations. MDS analyses were performed using each of the three marker classes separately and on the combined data set. Spatial variation was further summarized for the nuclear loci using a hierarchical likelihood ratio analysis (*G*-statistic, Sokal and Rohlf 1995). In a complimentary analysis for mtDNA, geographic heterogeneity at a series of hierarchical levels was assessed using Monte Carlo simulations (Roff and Bentzen 1989) with 10 000 replicate simulations.

Simulated mixed-stock assessment of U.S.–Canada allocations

The accuracy and precision of population allocations to simulated U.S.–Canada mixed-stock fisheries were evaluated. Simulations were performed using the Statistical Package for Analyzing Mixtures developed by the Alaska Department of Fish and Game using the GIRLS (Masuda et al. 1991) and CONJA-S (Pella et al. 1996) algorithms. Analyses were conducted for each marker class separately and on the combined data set. The program used both the iteratively reweighted least squares (Pella and Milner 1987) and expectation maximization (Pella and Milner 1987) maximum likelihood algorithms in simulations of possible mixture scenarios to evaluate the performance of MSA for the eight populations. The estimation procedure also includes parametric (Monte Carlo) bootstrapping to evaluate bias (accuracy) and variance (precision) due to mixture sampling error and to sampling error in the baseline. Sample sizes of 400 fish were used for each simulation. Estimates of precision were based on 100 bootstrap replicates, where both the mixtures and baselines were resampled each iteration and are presented for each marker class and for the combined analysis by 1 SD about the mean estimate. Simulations were run for each of six mixed-stock data sets, constructed so that the proportion of U.S. and Canadian reporting groups varied incrementally by 20% from 0% U.S. to 100% U.S.

The effects of differences in the relative abundance of stocks contributing to a mixture were evaluated using two population weighting strategies (equal and proportional weighting). Since researchers often lack information on stock size, we initially assumed that each population contributed equally to the simulated mixture. Simulations were also conducted weighting the population contributions to the mixture by estimates of escapement. Estimates of ecapement for each sampling area were 100 000 or 0.288 for the upper Tanana River (Delta), 58 600 or 0.169 for the Chandalar River, 86 400 or 0.249 for the Sheenjek River, 34 900 or 0.100 for Fishing Branch Creek, 58 600 or 0.169 for the Canadian mainstem (Tachun Creek, Big Creek, and Minto Slough), and 8700 or 0.025 for the Kluane River (L. Barton, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701, U.S.A., personal communication).

Previous data (Wilmot et al. 1992) suggested that populations of fall-run chum salmon could be grouped based on biological criteria (e.g., distance between populations, glacial history of spawning areas, bank orientation during migration) and that these groupings may confer greater accuracy and precision in MSA than have previously been realized using political boundaries (United States and Canada) as the sole criterion for establishment of reporting groups. To test alternative criteria, we grouped the eight populations into three reporting groups (Delta River, Kluane River, and the U.S.–Canada border and Canadian mainstem).

Finally, to assess alternative marker selection strategies, MSA simulations were conducted using the United States and Canada as reporting groups and using 10 loci that were selected using different criteria. Selection criteria included 10 loci with (*i*) the largest estimates of total gene diversity (H_t), (*ii*) the largest estimates of F_{st}, (*iii*) the largest among-population *G*-statistic, (*iv*) and five sets of 10 loci selected randomly without replacement. For all simulations, mean mixture estimates were derived from 100 simulations using a mixture composition of 100% Canadian populations, with each population contributing equally to the mixture.

Results

Levels of genetic diversity

Twenty four nuclear loci (18 allozyme loci, one gene intron, and five microsatellite loci) were polymorphic across the eight chum salmon populations (Table 1). Variation was also found within the ND5/6 coding region of mtDNA (Table 1). Levels of genetic variability in terms of allelic diversity and H_t varied among marker classes and among loci within each class (Table 1). Sixteen of 18 allozyme loci were characterized by two alleles, and for nine loci, the frequency of the common allele was >0.85 across all populations. In contrast, the nDNA loci were generally characterized by higher numbers of alleles (range 2–20) and allele frequencies that were not dominated by a single allele (Table 1). The ND5/6 region of mtDNA was also highly polymorphic with seven haplotypes resolved. Estimates of H_t (Table 1) were generally higher for nDNA loci (mean 0.592) than for allozyme loci (mean 0.250), although considerable heterogeneity was observed among loci within each marker class (Fig. 2). Levels of mtDNA haplotype diversity (0.647) were generally comparable with H_t values for the nDNA loci.

Population genotypic frequencies did not deviate from Hardy–Weinberg expectations for most loci. Six percent (12 of 192 tests) were significant at $\alpha = 0.05$, and no test was significant after nominal α levels were corrected for multiple comparisons (24 tests for each population), suggesting that variation at each nuclear locus was consistent with disomic inheritance at single loci. No indication of null alleles was observed at the microsatellite loci.

Spatial variation

Significant differences in allele frequency among the eight populations were observed for eight of 18 allozyme loci (mean $\theta = 0.010$, $P < 0.01$), four of six nDNA loci (mean $\theta = 0.011$, $P < 0.01$), and for mtDNA ($\theta = 0.016$, $P < 0.05$). No one marker class consistently exhibited higher levels of θ for all loci (Fig. 2). Although a larger proportion of the total genetic variation at the nDNA loci appears to be segregating within populations as compared with the allozyme loci, estimates of mean f, F, and θ were highly concordant. Estimates of θ were also similar to results from mtDNA. These results suggest that all three marker classes appear to capture the same signature of

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Table 1. Analysis of genetic diversity within and among eight populations of fall-run chum salmon from the Yukon River drainage.

Marker class	Locus	No. of alleles	Range of common allele frequencies[a]	*F*-statistics[a]			
				H_t[b]	*F*	*f*	θ
Allozymes	*sAAT-1,2**	2	0.935–0.971	0.079	0.018	0.019	0.000
	*sAAT-3**	2	0.543–0.678	0.472	0.164	0.164**	0.000
	*mAAT-1**	2	0.878–0.970	0.151	0.026	0.023	0.003
	*mAH-3**	2	0.667–0.924	0.362	–0.044	–0.075	0.028**
	*ALAT**	2	0.925–0.992	0.010	0.034	0.034	0.000
	*EST-D**	2	0.575–0.820	0.441	0.046	0.032	0.017*
	*G3PDH-2**	2	0.840–0.941	0.205	–0.018	–0.022	0.003
	*bGLU-A**	2	0.910–0.958	0.118	0.011	0.014	0.000
	*mIDHP-1**	2	0.980–1.000	0.011	–0.004	–0.008	0.004
	*sIDHP-2**	3	0.400–0.602	0.531	0.046	0.036	0.010*
	*LDHA-1**	2	0.432–0.726	0.457	–0.020	–0.036	0.015*
	*sMDHA-1**	2	0.899–0.958	0.131	0.025	0.020	0.004
	*sMDHB-1,2**	2	0.996–1.000	0.001	0.000	0.000	0.000
	*mMEP-2**	2	0.840–0.992	0.129	0.070	0.044	0.027**
	*MPI**	2	0.875–0.950	0.143	–0.054	–0.064	0.009*
	*PEPB-1**	3	0.742–0.892	0.266	0.069	0.061	0.008*
	*PEPLT**	2	0.942–1.000	0.046	–0.021	–0.031	0.010*
	*PGDH**	2	0.960–0.992	0.045	0.075	0.079	0.000
Mean ± SE	All loci			0.250	0.034	0.024±0.003*	0.010±0.003**
nDNA	GTHD*	3	0.463–0.663	0.540	–0.028	–0.030	0.002
	*One*μ1	2	0.630–0.930	0.370	0.005	–0.032	0.036**
	*One*μ10	6	0.367–0.493	0.668	0.003	0.004	0.000
	*One*μ18	3	0.570–0.735	0.495	0.027	0.019	0.009*
	*Ots*1	20	0.076–0.305	0.853	0.126	0.114**	0.013**
	*Ssa*14	12	0.440–0.730	0.622	0.026	0.010	0.016**
Mean ± SE	All loci			0.592	0.030	0.025±0.004	0.011±0.004**
mtDNA	ND5/6	14	0.063–0.556	0.647[c]		0.016*[d]	

Note: $*P < 0.05$; $**P < 0.01$.

[a]Allele frequency data for individual populations are available from the senior author upon request.

[b]Total gene diversity (Nei 1973) across the eight stocks.

[c]Haplotypic diversity (Nei and Tajima 1981).

[d]Estimates derived as described by Excoffier et al. (1992).

microevolutionary events that have given rise to the present spatial allelic diversity.

The eight populations could be combined into five groups (Delta River representing the Tanana drainage, Chandalar River, Sheenejk River, and Fishing Branch Creek on the Porcupine River, Kluane River, and the three populations from the Yukon River mainstem). Twelve of the 24 nuclear loci (cumulative $G = 517.55$, $P < 0.01$; Table 2) and mtDNA ($\chi^2 = 54.46$, $P < 0.05$) differed significantly among these five groups. Hierarchical analyses of heterogeneity in allele frequency revealed little evidence of significant spatial differentiation among populations of the same drainage (Table 2). Two of the 24 nuclear loci (*mAH-3** and *GH2D**) showed significant heterogeneity between the two populations from the Porcupine River (Sheenjek River and Fishing Branch Creek) at $\alpha < 0.05$, although none were significant when levels were adjusted for multiple testing. No evidence for spatial differentiation was noted among the three Canadian Yukon River mainstem populations (Table 2). Results of analyses of population variation in mtDNA haplotype frequency were concordant with those for both classes of nuclear loci. Significant differences were noted among groups ($\chi^2 = 46.92$, $P < 0.005$), although no significant differences were observed among populations within a drainage.

Interpopulation relationships based on genetic distance, when summarized in the form of MDS plots, revealed that interpopulation similarity is generally a function of geographic proximity (Fig. 3). This trend was consistent across the three marker classes. Populations from the Canadian mainstem cluster, as do the Sheenjek River and Fishing Branch Creek populations from the Porcupine River. Delta River and Kluane River were genetically distinct. The U.S.–Canada border population from the Chandalar River was more clearly distinguishable from the other border populations based on mtDNA.

Simulation analyses

Accuracy graphs were constructed depicting the accuracy and precision of simulation results of the relative abilities of each marker class to allocate simulated mixtures to each of two reporting groups (United States and Canada). Results of simulations conducted under assumptions of equal stock contribution (Fig. 4) and when stock contributions were weighted proportionally by estimates of escapement (data not shown) were very similar in terms of accuracy and precision. Analyses

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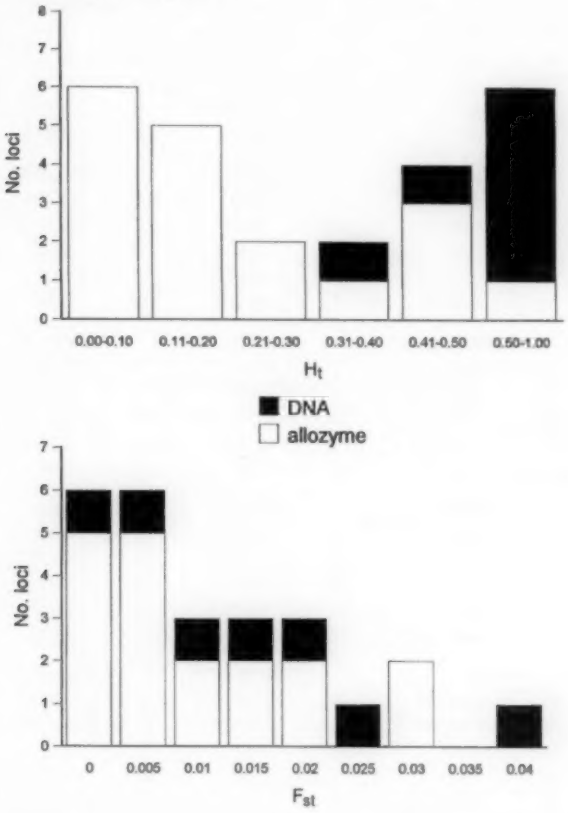
Fig. 2. Frequency histograms describing locus distributions of H_t and F_{st} values for allozyme, nDNA, and mtDNA markers from analyses of eight populations of fall-run chum salmon from the Yukon River drainage.



for each marker class revealed a consistent bias toward overallocation of Canadian stocks in simulated contributions up to about 50%. Greater precision was attained when data for all loci were included in the analysis (full data set, SD range 5–6%) relative to analyses conducted using each marker class separately (allozymes: SD 6.5–10%; nDNA loci: SD 6.5–9%; mtDNA: SD 17.5–22.5%). Bias for simulations conducted at 0, 20, and 40% expected Canadian contributions was due to underallocation of U.S. stocks (particularly Delta River) and overallocation of genetically similar Canadian border and mainstem stocks. Simulations conducted at 60% Canadian contribution were highly accurate. Estimates from simulations conducted at 80 and 100% Canadian contribution consistently underallocated fish to the Canadian border population from Fishing Branch Creek and overallocated fish to U.S. border populations from the Chandalar River and Sheenjek River populations.

Simulation analyses were conducted to examine accuracy and precision of reporting regions defined based on biological criteria. Analyses conducted with and without weighting revealed that individuals from the border and mainstem populations were allocated with high accuracy and precision to the border reporting group (Table 3). However, a high proportion of Delta River and Kluane River reporting groups were consistently misallocated to the border reporting group.

No clear locus selection criterion was resolved based on comparisons of mixed-stock simulations using a mixture composed of 100% of Canadian populations, with each population contributing equally to the mixture. Subsets of 10 loci that exhibited the highest levels of H_t (mean estimate ± 1 SD = 0.893 ± 0.052), highest estimates of F_{st} (0.893 ± 0.059), or highest *G*-statistics (0.882 ± 0.069) were no more accurate or precise in allocating stocks based on U.S. and Canadian reporting groups than were random draws of a comparable number of loci (ranges of mean estimates (0.895–0.900) and SDs (0.062–0.070)) or relative to simulation results when all 25 loci were used together (0.907 ± 0.041).

Discussion

Over the past decade, increasing attention has been focused on examination of genetic polymorphisms at the DNA level. Interest in this area has been motivated in part by the refinement of molecular techniques capable of resolving microevolutionary variation in genetic markers with contrasting modes of inheritance and rates of mutation and by the increased accessibility of technologies to fisheries biologists. Technological developments have given rise to a diverse array of molecular genetic markers (see review by Park and Moran 1994). However, while various molecular genetic markers have been used for chum salmon in several locations in the United States and Canada (e.g., mtDNA, Park et al. 1993; minisatellites, Beacham 1996) and for various other salmonid species (e.g., *Salmo* sp., Ferguson et al. 1995; Atlantic salmon (*Salmo salar*), Galvin et al. 1995; Tessier et al. 1995), few data exist that have simultaneously examined multiple molecular and allozyme loci to address questions of marker suitability for questions of population differentiation and fisheries assessment. As a result, fisheries researchers ask what criteria should be used to determine "the right marker for the right job"? Data drawn from different processes may have different sampling errors. If there is heterogeneity among data sets with respect to some property that affects estimation of population relationships, combining the data can lead to misleading results.

Comparisons among marker classes

Estimates of the extent of population differentiation (θ) were highly concordant between marker classes. Further, accuracy and precision from mixed-stock simulations were similar for allozymes and nDNA. We observed an increase in precision in simulated mixed-stock assessments when all markers were combined (Fig. 4), suggesting that all genetic markers appear to similarly track microevolutionary processes that have given rise to the observed patterns of genetic diversity. Loci with different inherent properties and rates of evolution can be used simultaneously in a GSI context. Secondly, results of simulations were entirely consistent regardless of the assumptions used to establish specific stock contributions to U.S. and Canadian reporting groups in the simulated mixture analysis (i.e., when stocks were assumed to contribute equally or when stocks were weighted in proportion to escapement estimates). Finally, political boundaries appear to poorly differentiate reporting groups when used in the allocation of mixed-stock fisheries. Although the degree of bias was not

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Table 2. Hierarchical heterogeneity analysis (modified from Smouse and Ward 1978) using allozyme and nDNA data collected for fall-run chum salmon in the Yukon River drainage.

Hierarchical level	df		df		df		df		df	
		*sAAT-1,2**		*sAAT-3**		*mAAT-1**		*mAH-3**		*ALAT**
Total	7	5.89	7	8.26	7	10.49	7	33.20***	7	9.31
Among	4	4.69	4	4.39	4	9.61*	4	27.78***	4	8.35
Within	3	1.19	3	3.86	3	0.87	3	5.41	3	0.94
Porcupine	1	0.00	1	3.36	1	0.10	1	3.99*	1	0.52
Canadian mainstem	2	1.19	2	0.50	2	0.77	2	1.42	2	0.42
		ESTD*		G3PDH-2*		bGLUA*		mIDHP-1*		sIDHP-2*
Total	7	21.34 **	7	9.86	7	5.13	7	10.30	14	27.77*
Among	4	18.96 ***	4	6.71	4	5.04	4	7.89	8	22.21**
Within	3	2.37	3	3.13	3	0.08	3	2.40	6	5.54
Porcupine	1	0.31	1	0.05	1	0.05	1	2.40	2	1.18
Canadian mainstem	2	2.06	2	3.08	2	0.03	2	0.00	4	4.36
		LDH-A1*		sMDH-A1*		sMDH-B1,2*		mMEP-2*		MPI*
Total	7	18.10 *	7	11.69	7	4.07	7	30.23***	7	13.98
Among	4	14.89**	4	4.44	4	2.87	4	24.25***	4	11.64*
Within	3	3.20	3	7.23	3	1.19	3	5.97	3	2.33
Porcupine	1	0.02	1	0.10	1	1.19	1	0.00	1	0.28
Canadian mainstem	2	3.18	2	7.13	2	0.00	2	5.97	2	2.05
		PEP-B1*		PEPLT*		PGDH*		GH2D*		Oneu1
Total	14	20.79	7	15.91*	7	3.84	14	27.04*	7	36.46***
Among	8	15.15	4	14.19**	4	3.45	8	14.47	4	32.23***
Within	6	5.63	3	1.71	3	0.37	6	12.56	3	4.22
Porcupine	2	3.18	1	0.58	1	0.34	2	8.17*	1	2.85
Canadian mainstem	4	2.45	2	1.13	2	0.03	4	4.39	2	1.37
		Oneu10		Oneu18		Ots1		Ssa14		Overall
Total	35	42.76	14	33.25**	56	130.84***	49	119.46***	31	649.97***
Among	20	31.10	8	29.78***	32	95.96***	28	107.40***	18	517.55***
Within	15	11.64	6	3.46	24	34.87	21	12.05	13	132.42
Porcupine	5	2.15	2	2.03	8	9.33	7	5.09	45	47.37
Canadian mainstem	10	9.49	4	1.43	16	25.54	14	6.96	90	85.05

Note: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

great at U.S.–Canadian frequencies in the midrange (i.e., 60%), which mirror actual values in the fisheries (about 60% U.S. composition over an entire season, Wilmot et al. 1992), greater accuracy and precision may be attained when reporting groups are based on biological (i.e., geographic proximity) rather than political criteria.

Degree of population differentiation: chum salmon as a model system

This study focuses on only a fraction of the geographic range covered by recent allozyme surveys for chum salmon (e.g., Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994). These studies have documented significant spatial heterogeneity in allele frequencies over regional and local scales and have emphasized the importance of geographic proximity and run-timing as important variables underlying observed spatial heterogeneity in allele frequency. Results of this study support earlier findings of genetic diversity in the Yukon River based solely on allozymes. Assays of nDNA and mtDNA variation reveal that populations of fall-run chum salmon in the Yukon River drainage have high levels of genetic variability (Table 1). Populations from five population groupings differed significantly in allele frequency (Table 2) and could be grossly separated into three general groups (Delta River, border and Canadian mainstem populations, and Kluane River; Fig. 3), as had been described earlier based on a more comprehensive population survey that only used allozyme data (Wilmot et al. 1992).

Several biological factors may provide the basis for the three population groupings. The Delta River is in a nonglaciated area. Bank orientation of this and other Tanana River spawning populations is on the south side of the Yukon River near the confluence of the Tanana River during upstream migration. The Delta River population is the latest spawning aggregation in the Yukon River (late October – late November). The border and Canadian mainstem of the Yukon River were also not glaciated. Populations that spawn in this region migrate along the northern bank of the Yukon River during upstream migration and spawn during late September – mid-October. The Kluane River population also migrate upstream along the north bank of the river and spawn during late October. Divergence of fish from the Kluane River could be a function of postglacial founder events and genetic drift. Alternatively, the Kluane River could have been of southeastern

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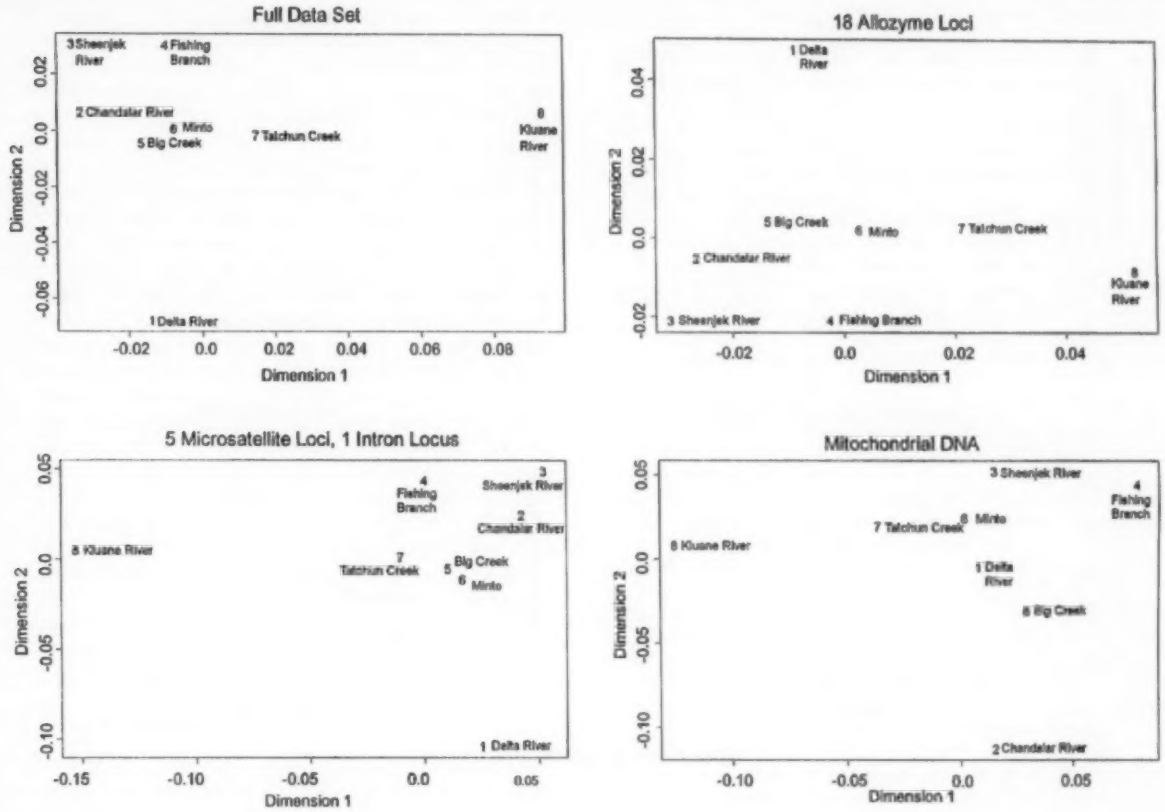
Fig. 3. Multidimensional scaling analysis comparing eight populations of fall-run chum salmon using the Cavalli-Sforza and Edwards (1967) chord distance.



Alaska origin owing to past stream capture events (Wilmot et al. 1994).

DNA analyses of chum salmon have not been conducted for summer-run stocks or for many of the fall-run chum populations. For eight populations of fall-run chum that have been surveyed intensively with several different genetic markers, results suggest that no single marker class is superior in discriminating among populations (Tables 1 and 2) or more accurate and precise in allocating stocks in a mixed-stock fishery (Table 3; Fig. 4). Allozymes and nDNA microsatellite and intron loci were similarly precise, and estimates of precision were highly concordant (Fig. 4). mtDNA alone was both imprecise and inaccurate (Fig. 4), although perhaps comparable results are likely any time a single locus is used. Greater precision was realized when all loci were combined in the simulated MSA.

Why intuitively might one marker class be better than another?

For rapidly evolving loci such as minisatellites and microsatellites, high rates of mutation may be an important factor contributing to patterns and levels of gene diversity within populations as well as variation among them. At equilibrium, the fixation index $F_{st} = 1/(4Nm + 1)$ where N is the effective population size and m is the rate of migration. Wright (1969) has shown that migration may be supplemented by reversible mutation (u) by substituting m with $(m + u)$. If mutation rates are high relative to the rate of gene flow among populations, then for neutral and highly mutable loci, novel mutations may contribute significantly to increased interpopulation variation. Conversely, if mutation rates are much lower than the rate of migration, F_{st} for neutral alleles has been shown to be nearly independent of mutation rate (Crow and Aoki 1984).

Given that markers may evolve at different rates, one data set may have an inordinately great influence on an analysis simply by virtue of having a larger number of character states. If different markers are evolving at different rates, and under the influence of different evolutionary forces, then estimates of population relationships (and statistical tests of significance) should consider a model of evolution that may vary among marker classes.

Various evolutionary processes could act upon each marker class differently. We assumed that the loci used for this study are selectively neutral. While few empirical or theoretical data have been presented for gene introns, recent literature on microsatellites has discussed that role of mutation in the estimation of population relationships based on genetic distance (Goldstein et al. 1995) and spatial variance in allele frequency (Slatkin 1995; see Scribner et al. 1996 for discussion of techniques and assumptions). Accumulating evidence (Scribner et

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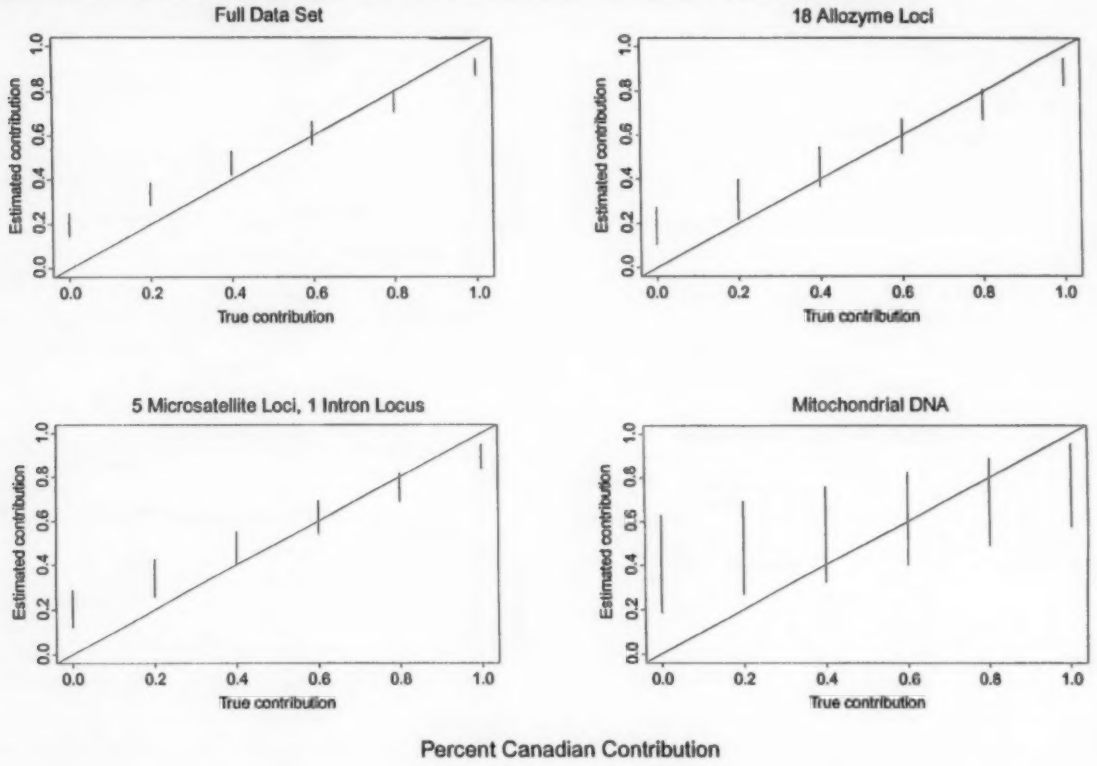
Fig. 4. Estimated (based on equal contributions of all populations) versus expected proportion of fall-run chum salmon in simulated mixed-stock fisheries. Estimates are the means of 100 bootstrap iterations and the error bars are 1 SD about the mean.



al. 1996; this study) suggests that for intraspecific comparisons among populations that over recent historical time have or continue to experience some level of gene flow, statistical analyses need not account for mutation rate heterogeneity. The number of generations separating populations is not likely to be sufficient to justify the incorporation of weighting strategies for microsatellites. Further, if microsatellite loci differ markedly in rates of mutation, this heterogeneity should be reflected in greater variances in F_{st}. Comparisons of the SEs about mean F_{st} estimates for microsatellites and allozymes (means ± SE were 0.011 ± 0.004 and 0.010 ± 0.003, respectively) do not show this trend.

In instances where few polymorphic allozyme loci are available for population-level analyses (e.g., *Salmo salar*, Galvin et al. 1995; Tessier et al. 1995), loci such as single-locus minisatellites and microsatellites, which are characterized by higher allelic diversities, would be expected to be comparatively more informative than allozymes. However, in situations where comparable numbers of polymorphic allozyme, minisatellite, or microsatellite loci are available, minisatellites and microsatellites would not be expected to exhibit greater levels of interpopulation variance in allele frequency. High levels of variation at these loci are a function of a high rate of mutation. As mutation rates affect only the probability of fixation and not the rate of fixation of neutral alleles, there is no greater likelihood for finding higher frequency stock-specific diagnostic markers at the hypervariable microsatellite or minisatellite loci than at polymorphic protein coding loci (Ferguson 1995). Our data support this concept. Estimates of interpopulation variance in allele (or haplotype) frequency were highly concordant across marker classes (Table 1). Further, marker selection cirteria based on preferential use of loci with high allelic diversity did not improve simulation estimates of population mixtures.

If no single class of genetic markers proves superior for

Table 3. Results from a simulation study evaluating biologically defined reporting regions.

Regional allocation	Mixture		
	Delta	Border	Kluane
Population contribution to border mixture weighted equally			
Delta	0.7972 (0.0707)	0.0213 (0.0183)	0.0033 (0.0071)
Border	0.1992 (0.0695)	0.9599 (0.0231)	0.1149 (0.0617)
Kluane	0.0010 (0.0026)	0.0127 (0.0108)	0.8642 (0.0666)
Population contribution to border mixture weighted by escapement			
Delta	0.7972 (0.0707)	0.0194 (0.0189)	0.0033 (0.0071)
Border	0.1992 (0.0695)	0.9646 (0.0236)	0.1149 (0.0617)
Kluane	0.0010 (0.0026)	0.0074 (0.0087)	0.8642 (0.0666)

Note: Mean mixture estimates (SD in parentheses) were derived from 100 simulations where each mixture (N = 400) is sampled from a single reporting region. All loci were used.

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stock discrimination and assessments of stock allocation, other factors must be considered in order to forward recommendations to managers. Factors include cost and ease of collection and analysis, versatility, and time and cost necessary to complete baselines necessary for the analyses of fisheries samples of relevance to managers. Further, if data are needed on an in-season basis, are all markers assayable in a time frame necessary to provide data needed for decisions on fisheries openings and closures? Sampling for genetic markers that are assayed using PCR-based technology can be accomplished using extremely small samples (e.g., scales or 10^{-3} g of tissue) acquired through nonlethal sampling. Samples can also be highly degraded, which allows analyses of old samples (e.g., museum specimens, scales saved for many years, spawned-out carcasses). Perhaps the biggest concern involves the time and expense required to accumulate existing baseline information to the point where the necessary sampling of background data (i.e., putative spawning aggregations) is complete and statistical methodologies have been rigorously tested. For chum salmon at the spatial scale examined, each marker class appears to provide the accuracy and precision necessary for MSA and for descriptive and hypothesis-oriented research. Given that comparable levels of polymorphism are expressed by each candidate marker class, the relative merits of any marker class for a particular analysis may ultimately be based on the availability of baseline data. Molecular genetic markers can be added to existing protein allozyme data baselines and used collectively in MSA.

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PERSPECTIVES

Predicting the identity and impact of future biological invaders: a priority for aquatic resource management

Anthony Ricciardi and Joseph B. Rasmussen

Abstract: The identification and risk assessment of potential biological invaders would provide valuable criteria for the allocation of resources toward the detection and control of invasion threats. Yet, freshwater biologists have made few attempts at predicting potential invaders, apparently because such efforts are perceived to be costly and futile. We describe some simple, low-cost empirical approaches that would facilitate prediction and demonstrate their use in identifying high-risk species from an important donor region: the Ponto-Caspian (Black, Caspian, and Azov seas) basin. This region is the source of several freshwater organisms already invading North America, including the zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena bugensis*), ruffe (*Gymnocephalus cernuus*), and round goby (*Neogobius melanostomus*). Based on a thorough literature review, we identify 17 additional Ponto-Caspian animals that have recent invasion histories and are likely to be transported overseas in ship ballast water; moreover, their broad salinity tolerance could allow them to survive an incomplete ballast-water exchange. These results suggest that, unless current vectors are more effectively controlled, the Great Lakes – St. Lawrence River system and other North American inland waterways will continue to receive and be impacted by invasive Eurasian species.

Résumé : L'identification des espèces susceptibles de devenir envahissantes et l'évaluation des risques qu'elles peuvent poser permettraient de définir des critères utiles pour l'affectation de ressources à la détection et à l'élimination des espèces nuisibles. Jusqu'ici, pourtant, les biologistes des eaux douces n'ont guère consacré de temps à cette question, de telles études paraissant être considérées comme coûteuses et inutiles. Nous décrivons ici des méthodes de prévision empiriques, simples et peu coûteuses et nous montrons comment les utiliser pour identifier les espèces à fort potentiel de nuisibilité d'une région qui peut être une importante source de ce genre d'organismes : le bassin pontocaspien (comprenant la mer Noire, la mer Caspienne et la mer d'Azov). C'est en effet de cette région que proviennent plusieurs des espèces qui ont déjà envahi les eaux douces nord-américaines et notamment la moule zébrée (*Dreissena polymorpha*), la moule quagga (*Dreissena bugensis*), la grémille (*Gymnocephalus cernuus*) et le gobie arrondi (*Neogobius melanostomus*). Dans une revue documentaire approfondie, nous avons relevé 17 autres espèces animales d'origine pontocaspienne qui ont récemment envahi d'autres régions et qui pourraient vraisemblablement être transportées jusqu'ici dans l'eau de ballast des bateaux arrivant d'outremer; de plus, comme ces espèces tolèrent des conditions de salinité très variées, elles pourraient survivre dans une eau de ballast incomplètement changée. Ces résultats donnent à penser qu'à défaut d'un contrôle plus rigoureux des vecteurs actuels, de nouvelles espèces envahissantes continueront d'être introduites d'Eurasie et de provoquer des dégâts dans le bassin des Grands Lacs et du Saint-Laurent, ainsi que dans d'autres bassins hydrographiques de l'intérieur des terres en Amérique du Nord.

[Traduit par la Rédaction]

Introduction

One of the most pervasive and ecologically damaging effects of human activities is the widespread movement of species beyond their natural range. In most countries, 10^2–10^4 nonindigenous species have been documented and their numbers are increasing (Lodge 1993). In North America, hundreds of exotic plants and animals have become established in aquatic habitats during this century (e.g., Mills et al. 1993*a*, 1996; Cohen and Carlton 1998). Biological invasions will continue to occur, particularly as expanding global trade increases the volume of flora and fauna that is shuttled from one geographic realm to another (e.g., in the ballast water of ships; Carlton and Geller 1993). The aquarium industry, for example,

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Table 1. Some hypothesized general attributes of invasive aquatic species (based on Ehrlich 1986; Groves and Burdon 1986; Lodge 1993; Morton 1979, 1997).

1.	Abundant and widely distributed in original range
2.	Wide environmental tolerance
3.	High genetic variability
4.	Short generation time
5.	Rapid growth
6.	Early sexual maturity
7.	High reproductive capacity
8.	Broad diet (opportunistic feeding)
9.	Gregariousness
10.	Possessing natural mechanisms of rapid dispersal
11.	Commensal with human activity (e.g., ship ballast-water transport)

has deliberately moved thousands of foreign species into North America, paving the way for many unintentional introductions (Welcomme 1984).

Typically, only a small proportion (<10%) of introduced species cause significant impacts (Williamson and Fitter 1996), but some of these have had enormous ecological and economic consequences (OTA 1993). Advance information on invasive and potentially harmful organisms would provide valuable criteria for control measures, such as quarantines and import restrictions (Groves and Burdon 1986; Willan 1987; Jenkins 1996), for allocating resources to maximize detection and for guiding decisions about proposed introductions (e.g., Townsend and Winterbourn 1992). Yet, despite the ecological and economic damage caused by exotics such as the sea lamprey (*Petromyzon marinus*), purple loosestrife (*Lythrum salicaria*), Eurasian watermilfoil (*Myriophyllum spicatum*), zebra mussel (*Dreissena polymorpha*), and others (OTA 1993; Mills et al. 1993*a*), there have been few formal attempts at forecasting unplanned invasions in North American freshwater habitats (but see Mandrak 1989; Mills et al. 1993*b*). This is likely the result of what Rejmanek (1996) described as a "widespread pessimism regarding the prospect of predicting which species are likely to become successful invaders." Such pessimism may have led administrators to ignore the warning of consultants contracted by the Canadian government in 1980 to study the occurrence of foreign organisms in ship ballast water; the consultants reported the transport of living veliger larvae to the Great Lakes in European vessels and predicted the establishment of the zebra mussel in North America (Bio-Environmental Services Ltd. 1981).

Clearly, the science and management of exotic species need to become more proactive to prevent or mitigate invasion threats. In this perspective, we propose that limited, but valuable predictions of the invasion success of different aquatic organisms can be made using documented information. We suggest a few simple, low-cost approaches that would facilitate the identification of potential future aquatic invaders.

Guidelines for identifying potential aquatic invaders

Because any organism can potentially expand its population when introduced into a favorable environment, it would be impractical to construct a comprehensive list of potential invaders based solely on environmental tolerances. A more cost-effective strategy is to identify species with exceptionally high invasion and impact potential. Using concepts currently being developed in both marine and terrestrial invasion biology (e.g., Forcella et al. 1986; Carlton 1996; Mack 1996; Rejmanek 1996), we suggest the following guidelines for such a selection process.

1. Identify potential donor regions and dispersal pathways of future invaders

An essential first step toward predicting invasions is the identification of a potential geographic donor region. Active donor "hot spots" may be identified by their disproportionate numerical contribution to the floras and faunas of various recipient regions using updated species lists (e.g., Mills et al. 1993*a*, 1996; Cohen and Carlton 1998). Regions with similar climates may have a higher potential of successfully exchanging species, although predictions based on this assumption have had varied success (Mack 1996). Potential donor regions may also be identified by large-scale shipping patterns. The global dispersal of aquatic organisms has been achieved primarily through transoceanic shipping (Williams et al. 1988; Carlton and Geller 1993), with ports acting as distribution hubs (Morton 1987; Willan 1987). Therefore, because the bulk of international trade is carried by ships, regions with growing economies should be considered as potential future donors of exotic species.

The presence of strong dispersal pathways and vector activity between the potential donor and target regions may indicate a high probability of future invasion. For example, repeated ballast-water discharges by European shipping using the St. Lawrence Seaway have resulted in the establishment of numerous species of exotic algae, invertebrates, and fishes in the Great Lakes; in fact, over 30% of all invasions in the Great Lakes have occurred following the opening of the Seaway (Mills et al. 1993*a*). Similarly, an increase in the net tonnage of ships arriving from East Asian ports over the past three decades has coincided with introductions of a variety of Asian invertebrates into Pacific North American estuaries (Cordell et al. 1992; Cohen and Carlton 1998). Therefore, Carlton (1996) suggested that an analysis of shipping traffic from regions containing known invaders such as the zebra mussel (e.g., the Great Lakes) or the Japanese crab *Hemigrapsus sanguineus* (e.g., Long Island Sound) could help predict where these species will next invade. While the probability of future North American invasions should be reduced by recent U.S. and Canadian regulations requiring oceanic exchange of ballast water, not all ships comply and incomplete ballast-water exchanges are common (Locke et al. 1993). Because organisms with saline-tolerant life stages could survive an incomplete ballast-water exchange and remain in the residual water or tank bottom sediments (Carlton 1985; Carlton and Geller 1993; Locke et al. 1993), it is likely that ships will continue to introduce new aquatic species into North America.

2. Select potential invaders using biological criteria

After having identified a potential donor region, a pool of probable invaders could be selected based on a general biological profile of invasive species. Some hypothesized attributes of successful aquatic invaders are listed in Table 1. Few of these generalizations have been confirmed by rigorous

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Table 2. Ponto-Caspian fauna established in the Great Lakes – St. Lawrence River system (data from Mills et al. 1993*a*; Witt et al. 1997; Zaranko et al. 1997).

Taxon	Species	Common name	Year of discovery	Mode of invasion
Cnidarians	*Cordylophora caspia*	Hydroid	1956	Unknown
Amphipods	*Echinogammarus ischnus*	Gammarid amphipod	1995	Ship ballast water
Bivalves	*Dreissena polymorpha*	Zebra mussel	1988	Ship ballast water
	Dreissena bugensis	Quagga mussel	1989	Ship ballast water
Fishes	*Cyprinus carpio*	Common carp	1879	Intentional introduction
	Gymnocephalus cernuus	Ruffe	1987	Ship ballast water
	Neogobius melanostomus	Round goby	1990	Ship ballast water
	Proterorhinus marmoratus	Tubenose goby	1990	Ship ballast water

statistical and experimental studies, and each has exceptions (Simberloff 1989; Lodge 1993); however, they apply well to a wide variety of invasive organisms and thus provide a useful basis for prediction. For example, a species with high genetic variability, wide tolerance limits, and natural mechanisms for rapid dispersal (e.g., the zebra mussel; Garton and Haag 1991) is more likely to colonize a large geographic range (Ehrlich 1986; Groves and Burdon 1986). Studies on herbaceous plants have shown that the size of the current distribution of a species is often a good predictor of invasiveness (Forcella et al. 1986; Roy et al. 1991; Rejmanek 1996). In fact, a large range is possessed by many invasive freshwater plants and animals, e.g., water hyacinth (*Eichornia crassipes*) (Groves and Burdon 1986), brown trout (*Salmo trutta*) (MacCrimmon et al. 1970), the cladoceran *Daphnia lumholtzi* (Havel et al. 1995), the hydrobiid snail *Potamopyrgus antipodarum* (Zaranko et al. 1997), and the Asiatic clam *Corbicula fluminea* (Morton 1997).

The most consistent attribute of an invasive species is human commensalism. Most successful invaders, particularly major pest species, use dispersal mechanisms that involve human activity (Ehrlich 1986; Groves and Burdon 1986; Morton 1979, 1997). Many invasive bivalves and crustaceans possess planktonic larvae that are more easily transported in ship ballast water (Morton 1979; Carlton 1985; Carlton and Geller 1993). Moreover, recent introductions of the amphipod *Echinogammarus ischnus* (Witt et al. 1997) and round goby (*Neogobius melanostomus*) (Jude et al. 1995) demonstrate that at least some organisms that lack a true planktonic stage can be transported by this mechanism. We recommend that profile-based predictions focus on the ability of an organism to exploit ballast-water transport and that particular attention be given to euryhaline organisms that are either planktonic (pelagic) or have planktonic life stages or behaviours.

3. Use invasion history as a predictive criterion

More reliable predictions can be made by assuming that invasive species will continue to invade elsewhere if conditions permit and opportunities arise (Daehler and Strong 1993; Carlton 1996). This premise can be combined with the previous profile-based approach by focusing on species that have an invasion history and an ability to interface with human-mediated dispersal mechanisms, particularly transoceanic shipping. Predictive power can be further enhanced by considering only species that have recently invaded sites (new potential donor regions) from which subsequent dispersal to the target region is more likely. Using this method, Mills et al. (1993*b*) successfully predicted the invasion of the Great Lakes by *P. antipodarum*, a snail with an extensive invasion history in Europe; it was recently reported from Lake Ontario (Zaranko et al. 1997). Similarly, *Limnoperna fortunei*, an Asian freshwater mussel that is currently expanding its range in both the Western Pacific and South America, is predicted to become established in North America (Ricciardi 1998).

Application to predicting invasions: the Great Lakes – Ponto-Caspian example

To demonstrate these approaches, we have selected the Great Lakes – St. Lawrence River as the target region and the Ponto-Caspian (Black, Caspian, and Azov seas) basin as a potential donor region. The former has been colonized by >140 exotic species, of which more than half are of Eurasian origin (Mills et al. 1993*a*; Witt et al. 1997; Zaranko et al. 1997). The Ponto-Caspian basin possesses a diverse endemic fauna (Mordukhai-Boltovskoi 1979*a*), is linked to North America by extensive shipping traffic (Locke et al. 1993; Mills et al. 1993*a*), and is the source of numerous invasive animals characterized by a broad ecological adaptability derived from having evolved under extremely variable hydrological and saline conditions (Zenkevitch 1963). Several Ponto-Caspian species are already established in the Great Lakes system (Table 2); with perhaps one exception, tubenose goby (*Proterorhinus marmoratus*), each of these has had a previous invasion history. Most (75%) were introduced by ballast water within the last 15 years. In fact, several ballast-water introductions of Eurasian fauna into the Great Lakes in the 1970s and 1980s had established a pattern of invasion prior to the arrival of the zebra mussel (Mills et al. 1993*a*). A focused search of other species likely to use this pathway would have identified the zebra mussel as a probable invader on the basis of its rapidly expanding European range, life history traits that facilitate ballast-water transport (i.e., planktonic larvae, wide salinity tolerance), and occurrence in estuarine waters where shipping traffic is frequent.

Using recent European literature (published after 1960) and Ponto-Caspian species lists provided by Mordukhai-Boltovskoi (1979*a*), we applied a combination of our predictive approaches to identify a pool of potential North American invaders. We considered 82 euryhaline macroinvertebrates that are endemic to freshwater regions of the Caspian Sea basin. From these, we selected species that belong to taxonomic

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Table 3. Ponto-Caspian fauna predicted to invade the Great Lakes – St. Lawrence River system.

Taxon	Species	Common name	Literature source[a]
Polychaetes	*Hypania invalida*	Polychaete worm	9, 10, 11, 14, 15, 16, 23
Amphipods	*Corophium curvispinum*	Corophiid amphipod	1, 4, 6, 7, 10, 11, 17, 19, 20, 21, 22, 23
	Corophium sowinskyi	Corophiid amphipod	7, 10, 11, 15
	Dikerogammarus haemobaphes	Gammarid amphipod	6, 9, 10, 11, 16
	Dikerogammarus villosus	Gammarid amphipod	10, 11, 13, 16
	Pontogammarus obesus	Gammarid amphipod	6, 7, 10, 11
	Pontogammarus robustoides	Gammarid amphipod	6, 10, 11, 16
	Obesogammarus crassus	Gammarid amphipod	6, 7, 10, 11
Mysids	*Limnomysis benedeni*	Mysid shrimp	10, 11, 12, 16
	Paramysis intermedia	Mysid shrimp	10, 11, 12, 18
	Paramysis lacustris	Mysid shrimp	3, 10, 11, 12
	Paramysis ullskyi	Mysid shrimp	10, 11, 12, 18
	Hemimysis anomala	Mysid shrimp	10, 11, 24
Bivalves	*Hypanys* (*Monodacna*) *colorata*	Cardiid clam	8, 10, 11, 12, 15, 23
Fishes	*Clupeonella caspia*	Tyulka; Caspian kilka	5, 10, 11, 12
	Benthophilus stellatus	Starry goby	10, 11, 12, 23
	Neogobius fluviatilis	Monkey goby	2, 10, 11, 12, 23

[a]Sources of information used in our literature survey: 1, Bachmann et al. 1997; 2, Biro 1972; 3, Bondarenko and Yablonskaya 1979; 4, Bortkevitch 1987; 5, Hoestland 1991; 6, Jazdzewski 1980; 7, Jazdzewski and Konopacka 1988; 8, Kogan 1970; 9, Lvova et al. 1996; 10, Mordukhai-Boltovskoi 1964; 11, Mordukhai-Boltovskoi 1979*a*; 12, Mordukhai-Boltovskoi 1979*b*; 13, Musko 1989; 14. Nagy 1979; 15, Pirogov et al. 1991; 16, Pligin and Yemel'yanova 1989; 17, Pygott and Douglas 1989; 18, Tarasov 1996; 19, Taylor and Harris 1986; 20, Tittizer et al. 1995; 21, van den Brink et al. 1993; 22, van der Velde et al. 1994; 23, Zenkevitch 1963; 24, Salemaa and Hietalahti 1993.

groups whose life history stages are likely to survive oceanic transport in ship ballast water (cf. Carlton 1985; Carlton and Geller 1993; Locke et al. 1993). From the resulting set (59 species), we identified those that had a documented invasion history, i.e., recent evidence of having successfully invaded habitats outside their natural range; for species that were introduced deliberately into new basins during stocking programs, we required evidence of unintentional secondary spread. Species that are currently invasive in Europe but that were judged unlikely to be transported in ship ballast water (e.g., the crayfish *Astacus leptodactylus*; Holdich and Reeve 1991) were rejected. Other groups (e.g., copepods, cladocerans) were not considered because of taxonomic inconsistencies or insufficient information on invasion history. Our survey (meant primarily to be illustrative rather than comprehensive) revealed 17 Ponto-Caspian invertebrates that have high invasion potential and are likely to survive an incomplete ballast-water exchange and thus should be considered as probable future immigrants to the Great Lakes (Table 3).

High-risk species: potential impact of Ponto-Caspian fauna

Can we predict the ecological impact of a Great Lakes' invasion by any of these species? An effective approach toward predicting the impact of an invader is to identify patterns in multiple, spatially independent invasions (e.g., Ricciardi et al. 1995, 1996, 1997; Grosholz and Ruiz 1996). While correlative analyses are often limited by a scarce amount of comparable quantitative data, descriptive information on the impacts of previous invasions may still provide a basis for useful predictions, although with a high degree of uncertainty (e.g., Grosholz and Ruiz 1996; Moyle and Light 1996). Simple, but powerful predictions may result by assuming that invaders will cause significant impacts in the target region if they have already done so in other regions (e.g., Forcella et al. 1986; Townsend and Winterbourn 1992; Ricciardi et al. 1995; Grosholz and Ruiz 1996). Arguably, this approach would have identified Eurasian invaders like the zebra mussel, ruffe (*Gymnocephalus cernuus*), and *Bythotrephes* as species of high impact potential prior to their arrival in North America. Therefore, we searched the literature for evidence of ecosystem alterations by our 17 Ponto-Caspian invertebrates or closely related species. Recognizing that these results are both speculative and subjective, our review suggests that each of the following species would have a significant ecological impact in the Great Lakes – St. Lawrence River system

Corophium spp.

The suspension-feeding amphipods *Corophium curvispinum* and *Corophium sowinskyi* inhabit fresh and brackish waters of lakes and rivers (Taylor and Harris 1986) and have been spreading across Europe since ca. 1900, using newly constructed canals and shipping traffic to enter adjacent river basins (Jadzewski 1980). Both species live in massive networks of mud tubes constructed on stable substrata. In the 1980s, *C. curvispinum* invaded the Rhine, colonizing hundreds of kilometres of the river and establishing enormous densities (100 000 – 750 000·m^{-2}) within only a few years (van den Brink et al. 1993). Similar high densities (>100 000·m^{-2}) have been recently recorded in other European rivers (Tittizer et al. 1995; Bachmann et al. 1997). By fixing thick layers (1–4 cm) of mud to the surfaces of stones, *C. curvispinum* displaced filter-feeding caddisflies (*Hydropsyche* sp.) and the zebra mussel from the Dutch Lower Rhine; zebra mussel larvae were

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denied suitable settling surfaces, and adult mussels were smothered to death by muddy encrustations (van der Velde et al. 1994). Competition for particulate food may also have occurred. Reductions in total organic carbon and suspended material in the lower Rhine have been attributed to the explosive population growth and filtration activity of the amphipod (van den Brink et al. 1993). These observations suggest that the introduction of Ponto-Caspian corophiids could significantly alter littoral communities and food webs in North American river systems.

Mysids

Mysid shrimp are diverse and abundant in shallow littoral areas of Ponto-Caspian deltas and estuaries (Mordukhai-Boltovskoi 1964, 1979*a*). A few species have demonstrated high invasion potential. Recognized as an important food item for fish, *Hemimysis anomala* and *Paramysis* spp. (*P. lacustris*, *P. intermedia*, *P. ullskyi*) have been intentionally transplanted, with remarkable success, into numerous lakes and reservoirs in Eurasia and the Baltic Peninsula, in which they have rapidly formed dense populations (Mordukhai-Boltovskoi 1964, 1979*a*). Another mysid, *Limnomysis benedeni*, was accidentally introduced into Dnieper reservoirs (Pligin and Yemel'yanova 1989) and has also spread 1700 km up the Danube River. These species have continued to expand their ranges in recent decades (Mordukhai-Boltovskoi 1979*a*, 1979*b*; Salemaa and Hietalahti 1993; Tarasov 1996).

Ponto-Caspian mysids differ strikingly from their North American relatives by their adaptation to shallow, warm waters; for example, the metabolic rate of *P. lacustris* at 20°C is similar to that of cold-water mysids at 10°C (Bondarenko and Yablonskaya 1979). This being the case, *Paramysis* and others could potentially invade a large number of North American lakes that are currently devoid of mysids. Their impact in these lakes would likely include a reduction of zooplankton (with concomitant negative effects on planktivorous fishes and higher consumers; cf. Spencer et al. 1991) and biomagnification of contaminants at higher trophic levels through a lengthening of the food chain; PCB and mercury levels in pelagic fishes have been shown to be higher in North American lakes containing mysids than in mysid-free lakes (Rasmussen et al. 1990; Cabana et al. 1994).

Clupeonella caspia

Three fishes from the Ponto-Caspian region, the ruffe, round goby, and tubenose goby, were introduced into the Great Lakes by ballast-water discharge in the 1980s (Mills et al. 1993*a*; Jude et al. 1995). Additional species that may arrive in the future include a Caspian herring, the "tyulka" (*Clupeonella caspia*). This pelagic planktivore is a fast-growing, eurythermal, and euryhaline species that has historically inhabited coastal waters of the Black and Caspian seas, but has expanded its range throughout the Volga, Don, and Kama rivers in recent decades (Hoestland 1991). In Volga River reservoirs, the tyulka is reported to have become abundant and suppressed native fishes (Mordukhai-Boltovskoi 1979*b*). It is capable of extreme population fluctuations, and mass winter mortalities in reservoirs have been reported (Hoestland 1991). We predict that the tyulka would cause reductions of native planktivores (e.g., coregonids, shiners, yellow perch (*Perca flavescens*)) in the Great Lakes system, similar to the observed impacts of another introduced clupeid, the alewife (*Alosa pseudoharengus*) (Brandt et al. 1987). The tyulka poses the added threat of invading lakes that are currently devoid of important pelagic forage fish, which could result in increased contaminant levels in piscivores, an effect already documented for introduced smelt (Vander Zanden and Rasmussen 1996).

Conclusion: using predictive approaches as an early-warning system

We have demonstrated how simple, low-cost approaches could be used to identify species with high invasion and impact potential. In spite of any perception that "the worst is over" (i.e., all significant invaders have already arrived), our results suggest that the Great Lakes – St. Lawrence River system and other North American inland waterways will continue to receive and be impacted by invasive Eurasian fauna.

Because the ecological and economic costs of invasion are high (OTA 1993), resources should be allocated towards prevention. Preventative measures depend on advance knowledge of invasion threats, which can be gained from careful monitoring of information networks (e.g., literature, the internet, symposia), and the application of the guidelines we have described. Such endeavors would be aided greatly by the development of a broadly accessible electronic inventory of aquatic invaders, with information about their life history, habitat requirements, dispersal patterns, and methods of control. Even when invasion is imminent, advance knowledge could prepare us for dealing with the ecological and technological impacts that may follow. Given the detrimental effects of invasive aquatic organisms on fisheries, biodiversity, outdoor recreation, and water supply systems (e.g., Morton 1979, 1997; Kaufman 1992; Lodge 1993; Mills et al. 1993*a*; OTA 1993), the identification of future invaders should be a common priority among scientists and policy makers.

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Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod?

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Abstract: The assumption that spawner biomass is directly proportional to total egg production by fish stocks underlies most spawner–recruit relationships. Despite its importance, this assumption is largely untested. Data describing the reproductive potential of Northeast Arctic (NEA) Atlantic cod (*Gadus morhua*) were used to test the proportionality assumption for a period showing strong variation in condition and abundance of spawners. At the individual scale, relative fecundity varied with length and condition. This decreases the likelihood that the proportionality assumption is valid. At the stock level, total egg production was estimated (1985–1996) using acoustic estimates of total abundance and demographic information from trawl sampling in combination with year-specific fecundity–length relationships. For NEA cod, spawner biomass estimated by virtual population analysis (VPA) was not proportional to total egg production. Compared with VPA-based estimates of spawner biomass, total egg production is an improved index of recruitment potential because (*i*) the magnitude of variation observed in total egg production was closer to that observed in recruitment and (*ii*) the relationship between recruitment at age 1 and total egg production approaches the origin. The results suggest that further progress in explaining recruitment variation will be achieved using more sensitive measures of the true reproductive potential of the stock.

Résumé : La plupart des relations géniteurs–recrutement décrites jusqu'ici sont fondées sur l'hypothèse que la biomasse des géniteurs d'un stock de poissons donné est directement proportionnelle à la ponte totale du stock en question. Or, malgré l'importance qu'elle revêt, cette hypothèse de proportionnalité n'a essentiellement pas été vérifiée. Pour vérifier si la relation de proportionnalité est fondée, nous avons appliqué des données relatives au potentiel de reproduction de la morue franche (*Gadus morhua*) du nord-est de l'Arctique (NEA) à une période où l'état et l'abondance des géniteurs ont présenté une forte variation. À l'échelle individuelle, nous avons constaté que la fécondité relative varie en fonction de la longueur et de l'état du poisson. Cette constatation vient diminuer la probabilité que l'hypothèse de la proportionnalité soit fondée. Pour estimer la ponte totale à l'échelle du stock pour la période de 1985 à 1996, nous avons utilisé l'estimation de l'abondance totale, déterminée par des relevés acoustiques, et l'information démographique obtenue par des échantillonnages au chalut, en combinaison avec les relations fécondité–longueur par année. Dans le cas de la morue franche NEA, la biomasse des géniteurs, estimée par analyse des populations virtuelles (APV), n'était pas proportionnelle à la ponte totale. Nous pensons que, contrairement à la biomasse des géniteurs estimée par APV, la ponte totale est un indice valable du potentiel de recrutement et ce, pour les raisons suivantes : (*i*) par son ampleur, la variation de ponte totale observée était plus proche de la variation de recrutement observée et (*ii*) la relation entre le recrutement de la classe d'âge 1 et la ponte totale est proche de l'origine. Les résultats de ces travaux permettent de penser que nous pourrions en venir à mieux comprendre la variation du recrutement en utilisant des paramètres plus sensibles pour mesurer le véritable potentiel de reproduction des stocks.

[Traduit par la Rédaction]

Introduction

The major outstanding question in the population dynamics of marine fish stocks concerns the existence of a relationship between the abundance of spawners and the number of recruits produced (Hilborn and Walters 1992). A metaanalysis of spawner–recruit relationships concluded that there is a central tendency for recruitment to be positively correlated with spawner biomass (Myers and Barrowman 1996). Even this general conclusion has been challenged (Gilbert 1997), prompting yet another round (Myers 1997; Francis 1997; Hilborn 1997) in a seemingly endless debate. From a management perspective, within-stock relationships are usually too weak to precisely define the biological reference points needed to develop optimal harvesting strategies (Sparholt 1995). This has led researchers to seek other correlates of recruitment (Shepherd et al. 1984; Wooster and Bailey 1989) and to advocate the development of management strategies that do not assume an underlying spawner–recruit relationship (Evans and Rice 1988; Koslow 1992). The situation has caused Rothschild (1986, p. 102) to ask: "How can there be so little evidence for

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Table 1. Cod stocks for which both VPA-based SB and field-based estimates of E are available.

Stock	Year	VPA-based SB (t)	E ($\times 10^{12}$)	Source of E estimate
NEA	1984	259 000	18.7	Sundby and Bratland 1987
	1985	201 500	21.4	
North Sea	1976	167 000	10.6	Harding et al. 1978
	1987	89 000	9.2	Heessen and Rijnsdorp 1989
	1988	85 000	10.5	
Irish Sea	1995	4 400	3.0	Anonymous 1997*c*

Note: VPA-based estimates of SB for NEA, North Sea, and Irish Sea cod were obtained from Anonymous (1997*a*, 1996, 1997*b*), respectively.

a relation of recruitment to parent stock when the very existence of such a relationship is so critical to population stability?"

Several explanations have been put forward to account for this apparent paradox. The most common is that the underlying spawner–recruit relationship is obscured by mortality during the prerecruit period (Goodyear and Christensen 1984; Cushing 1988; Walters and Collie 1988). It has also been suggested that fitting statistical models to the spawner–recruit relationship is complicated by the presence of outliers (Chen and Paloheimo 1995), by the fact that both the dependent and independent variables are measured with considerable error (Walters and Ludwig 1981), and (or) by time series effects that bias the parameter estimates (Walters 1985). These explanations all assume that there is a functional relationship between spawner biomass and recruitment and that the problem lies in extracting a signal of unknown strength from noisy time series data.

An additional possibility is that the weakness of spawner–recruit relationships results from a misspecification of the functional relationship. In the original theoretical formulations (see figs. 1–8 in Ricker 1954 and eq. 6.10 in Beverton and Holt 1957), recruitment was assumed to be dependent on total egg production by the stock. However, both studies initiated the practice of treating total egg production and spawner biomass as interchangeable quantities. For example, Beverton and Holt (p. 62) formulated total egg production (E) as

$$(1) \quad E = s\chi\mathrm{SB}$$

where s is the proportion of females, χ is the fecundity per unit of body weight (i.e., the relative fecundity), and SB is the spawner biomass. Both s and χ were treated as constants giving a direct proportionality between E and SB, although Beverton and Holt acknowledged that departures from proportionality were possible given that "survival of fry hatched from large eggs may be better than from small ones" (see footnote on p. 62).

The majority of spawner–recruit relationships have used estimates of SB generated using virtual population analysis (VPA) as the independent variable rather than E. Exceptions include stocks where spawners are not amenable to enumeration and synoptic-scale egg surveys are conducted to estimate total egg abundance (or production) as a proxy for SB (Lockwood et al. 1981; Lasker 1985; Lo et al. 1992). Another approach is to estimate E from the abundance of mature females by age in combination with fecundity–age relationships. For example, Serebryakov (1990) used VPA-based estimates of total abundance-at-age to estimate E for Northeast Arctic (NEA) Atlantic cod (*Gadus morhua*). Ideally, testing the assumption of proportionality requires that SB and E be estimated independently of each other. While there are a few cod stocks for which both VPA-based SB and estimates of E from egg surveys are available (Table 1), the number of years for which values of E are available is usually too low to give a powerful test of the proportionality assumption.

A review of the proportionality assumption as it applies to long-lived gadoids such as cod is timely for several reasons. At the individual level, there has been an increase in knowledge of the links between female characteristics of gadoids and the quantity and (or) quality of progeny (Hislop et al. 1978; Hislop 1988; Kjesbu et al. 1991, 1996; Karlsen et al. 1995; Koslow et al. 1995; Solemdal et al. 1995; Marteinsdottir and Steinarsson 1996). These links are often referred to as maternal effects (see recent reviews by Bernardo 1996 and Chambers and Leggett 1996) and there is growing recognition that this information is of utility in fisheries management (Ulltang 1996; Solemdal 1997; Trippel et al. 1997*a*). At the stock level, there is evidence that age diversity in the spawning stock is positively correlated with recruitment for Icelandic cod (Marteinsdottir and Thorarinsson 1998). The inferred mechanism underlying this correlation is that female characteristics that are likely to affect the reproductive potential of the stock (number of old/large fish, timing/duration of spawning) potentially influence recruitment via interannual variation in total egg production (quantity and quality). A corollary of this is that VPA-based SB is not a sensitive measure of total egg production given the lack of relationship between VPA-based SB and recruitment that is observed for the Icelandic cod stock.

The goal of this paper is to explicitly test the assumption that the VPA-based SB is proportional to E for NEA cod. NEA cod are well suited to testing this hypothesis by virtue of having detailed information describing size-dependent variation in reproductive potential (Solemdal 1970; Kjesbu et al. 1998) supported by additional information on Atlantic cod generally (Kjesbu et al. 1991, 1992, 1996; Solemdal et al. 1995). Combined acoustic and trawl surveys of the Barents Sea and spawning grounds off the Lofoten Islands provide acoustic estimates of total abundance that can be used to scale E independently of the VPA-based SB, while information from trawl sampling can be used to describe the demographic composition (size, sex, and maturity stage) of the stock. Lastly, the time period considered for the analysis includes a strong signal in both stock abundance and growth rates that was coincident with the collapse of the Barents Sea capelin (*Mallotus villosus*) stock in the late 1980s (Jakobsen 1993; Nakken 1994).

The approach taken was to examine the proportionality assumption at both the individual and stock levels for the time period covered by the surveys (1985–1996). Length-specific weight and the hepatosomatic index (HSI) were used to describe interannual variation in the physiological condition of individual spawners whereas fecundity and relative fecundity (χ) were used to describe their reproductive potential. At the stock level, data from combined acoustic/trawl surveys were used to estimate E. These estimates were then contrasted with VPA-based SB to test the proportionality assumption. To determine which was the better index of recruitment potential,

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Table 2. Codes used in the visual assessment of maturity stage for female NEA cod from 1988 to the present.

Stage	Description
1	Immature: small, reddish, clear ovary
2	Maturing: ovary is large in volume and firm; developing oocytes easily seen through the ovarian wall; no clear, large oocytes (eggs)
3	Spawning: light pressure releases eggs or eggs spread around in the ovary soon ready to be spawned
4	Spent: ovary is small, loose, and bloody
5	Uncertain

Table 3. Summary data for total wet weight–length regressions for prespawning female NEA cod.

Year	Area	n	r^2	a	b
1986	Vesterålen	56	0.98	0.0017	3.37
1987		23	0.91	0.0002	3.87
1988	Barents Sea	36	0.95	0.0150	2.85
1989		68	0.97	0.0091	3.00
1990		33	0.96	0.0113	2.96
1991		162	0.91	0.0156	2.89
1992		153	0.91	0.0080	3.03
1993		143	0.94	0.0030	3.26
1994		84	0.97	0.0037	3.20
1995		102	0.96	0.0046	3.14
1996		92	0.95	0.0055	3.13

Note: The independent and dependent variables were transformed to common logarithms, and then, simple linear regression was used to estimate the slope and intercept. Values of a and b given correspond to the following model: weight (g) = a·length (cm)^b.

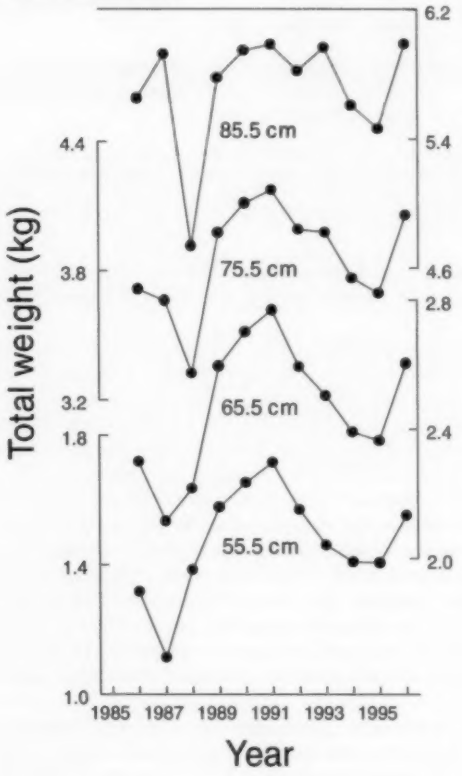
the relationship between E and abundance at age 1 was contrasted with the conventional relationship between the VPA-based SB and abundance at age 1. The results are discussed with reference to current management protocols for NEA cod.

Interannual variation in condition

Length-specific weight is an easily computed measure of condition that is representative of the growth history of fish (Winters and Wheeler 1994). Data from Norwegian surveys of the Barents Sea conducted in January–March (Jakobsen et al. 1997) were used to develop year-specific weight–length regressions for prespawning females from 1988 to 1996. Prespawning females were defined as those females classified as maturity stage 2 (Table 2). Because the surveys did not consistently record individual weight prior to 1988, data collected for prespawning females sampled off the coast of Vesterålen (located just north of Lofoten; see Kjesbu (1994) for map) were used to develop year-specific weight–length regressions for 1986 and 1987. Weight data for 1985 are not available. Summary statistics for the weight–length relationships are given in Table 3. These relationships were used to estimate total body weight for four standard lengths (55.5, 65.5, 75.5, and 85.5 cm). These four standard lengths encompass the majority of the population of prespawning females and correspond to the midpoints of the 10-cm length-classes used to describe liver condition (see below).

The minimum total body weight for 55.5- and 65.5-cm cod

Fig. 1. Time series (1986–1995) of total body weight of 55.5-, 65.5-, 75.5-, and 85.5-cm prespawning female NEA cod estimated using the regressions in Table 3. The vertical scales are discontinuous so that variation in one length can be viewed relative to variation in the other three.



was observed in 1987, 1 year before minimum values were observed for 75.5- and 85.5-cm cod (Fig. 1). The ratio of minimum to maximum weight ranged from 0.65 for 55.5-cm cod to 0.80 for 85.5-cm cod, indicating that in a poor-condition year the total body weight of prespawning females was between 65 and 80% of the total body weight in a good-condition year.

The HSI is a sensitive index of the energetic reserves of cod (Lambert and Dutil 1997). These data have been collected for NEA cod by Russia on a monthly basis since 1927 (Yaragina 1996). Since 1967, the data have been summarized by 10-cm length-class (21–30 through to 91–100 cm) and region (southern Barents Sea and Svalbard region). The HSI values presented here are a subset of the complete database in the following respects: (*i*) only values for cod in the length-classes 51–60, 61–70, 71–80, and 81–90 cm are presented, as these four length-classes constitute the majority of the spawning population and (*ii*) only values for cod sampled in the southern Barents Sea are included (i.e., data for cod sampled in the Svalbard region are not presented, as these individuals are assumed to constitute a minor subcomponent of the stock). Observations for males and females were pooled, although systematic differences between sexes have been noted for cod larger than 60 cm (Yaragina 1992).

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Table 4. Number of liver weight observations for NEA cod (southern Barents Sea only) by month and year.

Length-class (cm)	Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
41–70	1985	125	95	0	0	73	531	349	359	0	0	0	715	2247
	1986	614	456	729	266	237	466	792	738	94	57	94	528	5071
	1987	89	80	96	174	88	245	688	95	624	376	548	450	3553
	1988	437	0	181	0	114	163	300	501	232	631	279	250	3088
	1989	308	344	95	109	0	198	504	257	312	171	440	299	3037
	1990	148	73	0	37	0	391	136	281	129	275	258	376	2104
	1991	0	0	92	225	170	376	0	216	0	159	274	22	1534
	1992	69	0	294	0	230	71	0	0	0	0	313	131	1108
	1993	85	75	300	1190	936	178	499	390	95	157	181	142	4228
	1994	40	137	0	529	507	93	451	110	410	480	353	424	3534
	1995	854	106	957	455	238	303	417	0	409	183	502	903	5327
	1996	533	256	548	420	803	988	316	107	308	855	741	282	6157
71–100	1985	74	52	0	0	27	89	49	39	0	0	0	49	379
	1986	37	38	62	30	31	76	27	58	2	42	4	17	424
	1987	1	18	4	4	12	2	50	27	23	7	35	44	227
	1988	12	0	18	0	4	19	17	46	14	25	8	7	170
	1989	29	28	5	1	0	6	66	3	34	64	89	56	381
	1990	12	27	0	12	0	108	8	12	68	180	179	202	808
	1991	0	0	15	100	240	201	0	71	0	65	182	12	886
	1992	29	0	119	0	93	23	0	0	0	0	211	109	584
	1993	21	24	56	120	346	106	122	70	4	47	114	189	1219
	1994	17	77	0	304	131	7	159	20	76	85	84	178	1138
	1995	270	54	209	105	108	71	49	0	28	16	107	277	1294
	1996	130	52	150	48	225	339	124	26	62	119	266	203	1744

Note: Information given is for aggregated length-classes (41–70 and ≥71 cm) rather than 10-cm length-classes. Note that it was not possible to partition the number of observations into 10-cm length-classes. Annual totals are given.

The HSI was estimated as follows:

$$\text{(2)}\quad \text{HSI} = \frac{\sum_{n} \text{liver wet weight}}{\sum_{n} \text{total wet weight}} \times 100\,(\%)$$

where n represents the total number of observations in each year, month, and length-class. The number of observations in each month is given by year in Table 4 to indicate interannual variation in sampling effort. For each of the four length-classes the arithmetic mean of monthly HSI values ($\overline{\text{HSI}}_{\text{Jan.–Dec.}}$) was estimated. Given that there is a strong seasonal cycle in HSI values (Yaragina 1996), $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$ will be affected by variation in the frequency of missing values of HSI. For the four 10-cm length-classes used here, 102 out of 576 (17%) of the monthly HSI values over the time period 1985–1996 were missing. Missing values were replaced by linearly interpolated values prior to estimating $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$.

Values of $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$ varied between 3.3 and 7.8% from 1985 to 1996 (Fig. 2A). The temporal trends for all four length-classes are quite synchronous. The dominant signal in the time series is a decrease from 1983 to minimum values in 1987 followed by an increase to maximum values in 1991. A second decrease in $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$ occurred from 1991 to 1995, while a slight increase in 1996 is indicated. The interannual variation in $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$ parallels the variation in capelin biomass (Fig. 2B) and in length-specific weight (Fig. 1). The association between cod liver condition and capelin abundance has been noted previously for NEA cod (Nilssen et al. 1994; Yaragina 1996) and for cod in the Newfoundland–Labrador region (Taggart et al. 1994; Lilly 1996). Cod in poor condition having lower HSI values are more likely to have reduced fecundity, increased frequency of atresia, and possibly smaller eggs in general (Kjesbu et al. 1991). Thus, the reproductive potential of individual NEA cod might be expected to covary with variation in $\overline{\text{HSI}}_{\text{Jan.–Dec.}}$.

Interannual variation in fecundity

Fecundity is a direct measure of the reproductive potential of individual fish. It is useful to distinguish between two different measures of fecundity. "Potential fecundity" refers to the number of developing (or vitellogenic) oocytes in the prespawning ovary that can potentially be shed. "Realized fecundity" refers to the actual number of eggs that are produced by a fish in one season once factors such as atresia are accounted for (cf. Ma et al. 1998). The term "fecundity" as used here refers to potential fecundity unless specified otherwise. Fecundity was determined for prespawning NEA cod sampled off of Vesterålen in 1986, 1987, 1988, 1989, and 1991. Fortuitously, these five years span the maximum range in variation in liver condition (Fig. 2A). As a full description and analysis of the fecundity data is given in Kjesbu et al. (1998), only information that is relevant to this study is presented here.

For 1986–1989, only prespawning females were sampled for fecundity. Data for 1991 include observations for both prespawning ($n = 9$) and spawning ($n = 46$) females (Table 5). Fecundity for the latter group was corrected for the proportion of eggs spawned as described by Kjesbu et al. (1998). Relative

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Fig. 2. (A) Time series (1985–1996) of the $\overline{HSI}_{Jan.-Dec.}$ for NEA cod in length-classes 51–60, 61–70, 71–80, and 81–90 cm. The arrows on the *x*-axis indicate years where fecundity samples were collected off Vesterålen. (B) Time series (1985–1996) of total stock biomass of Barents Sea capelin from table 4.3.3 of Anonymous (1997*d*).

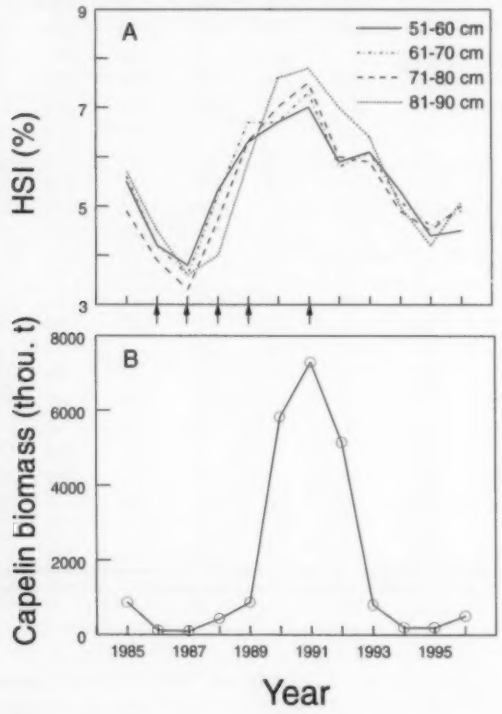

Table 5. Summary data for fecundity–length regressions for prespawning female NEA cod.

Year	Length range (cm)	*n*	r^2	*a* ($\times 10^{-7}$)	*b*
1986	55–135	50	0.89	1.23	3.76
1987	50–86	46	0.79	2.61	3.54
1988	50–122	49	0.92	0.61	3.91
1989	50–126	111	0.92	8.05	3.36
1991	56–127	55	0.64	74.67	2.98

Note: Both the independent and dependent variables were transformed to natural logarithms, and then, simple linear regression was used to estimate the slope and intercept. Values of *a* and *b* given correspond to the following model: fecundity (no. $\times 10^6$) = a·length (cm)^b.

fecundity for individual cod (χ) was calculated by dividing the fecundity by total body weight. The length range in 1987 was very restricted compared with the other four years (Table 5) because no females larger than 86 cm were captured that year during the sampling program in Vesterålen. Consequently, 1987 was not included in the statistical analysis of the effect of length on χ. Data for 1991 were not used to estimate χ because the observations for spawning females could not be corrected for changes in total body weight that occur during spawning.

The assumption of proportionality between SB and *E* is more likely to be valid if the relative fecundity of individuals (χ) is constant both with length and among years (eq. 1). This is not the case (Fig. 3). Analysis of covariance indicated that, in addition to the significant effect of length on χ ($p = 0.0001$), there are significant interannual differences ($p = 0.001$) in the intercept of the χ–length relationship that can be attributed to interannual variation in condition. For this analysis, condition was represented by the HSI value for 61- to 70-cm cod in the previous year ($\overline{HSI}_{Jan.-Dec.,t-1}$), as it would be more representative of energy reserves available for gonadal maturation and spawning. The positive correlation between χ and length means that large cod are more fecund per gram body weight (≈500 eggs·g^{-1}) compared with small cod (≈300 eggs·g^{-1}). This result is important from a population dynamics perspective because it becomes more likely that a stock composed of small cod will produce fewer eggs than the equivalent biomass of large cod, a direct violation of the proportionality assumption. Many of the heavily exploited cod stocks, including NEA cod (Nilssen et al. 1994), have undergone substantial reductions in the number of large fish (Trippel et al. 1997*a*). The significant effect of condition on both χ (noted above) and

Fig. 3. Relationship between length and relative fecundity (χ = fecundity·total body weight^{-1}) for NEA cod in (A) 1986, (B) 1988, and (C) 1989. Analysis of covariance indicated a significant effect of both length and condition on χ ($r^2 = 0.22$, $n = 209$), although the interaction term (length × $\overline{HSI}_{Jan.-Dec.,t-1}$) was nonsignificant ($p = 0.17$).

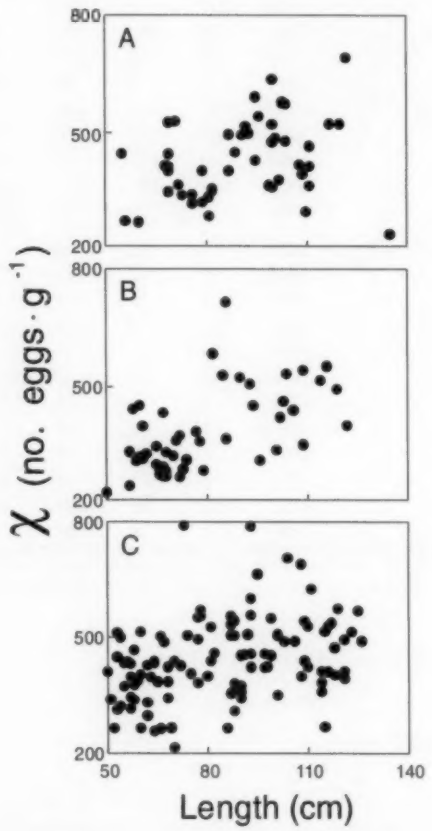

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Table 6. Total acoustic abundance for Norwegian acoustic surveys in the Barents Sea (January–March) and Lofoten area (March).

Year	Area	Total abundance ($\times 10^6$)	Source
1985	Barents Sea	1218	Table A.2 in Anonymous 1997*a*
1986		2676	Table A.2 in Anonymous 1997*a*
1987		487	Table A.2 in Anonymous 1997*a*
1988		224	Table A.2 in Anonymous 1997*a*
1989		174	Table A.2 in Anonymous 1997*a*
1990		526	Table A.2 in Anonymous 1997*a*
1991		591	Table A.2 in Anonymous 1997*a*
1992		1201	Table A.2 in Anonymous 1997*a*
1993		2688	Table A.2 in Anonymous 1997*a*
1994		2685	Table A.2 in Anonymous 1997*a*
1995		3570	Table A.2 in Anonymous 1997*a*
1996		3089	Table A.2 in Anonymous 1997*a*
1985	Lofoten	16.7	Corrected value from Godø et al. 1985
1986		10.4	Corrected value from Raknes and Sunnanå 1986
1987		15.9	Corrected value from Godø et al. 1987
1988		7.0	Corrected value from Hylen et al. 1988
1989		10.3	Corrected value from Jakobsen et al. 1989
1990		40.7	Table A.4 in Anonymous 1997*a*
1991		56.3	Table A.4 in Anonymous 1997*a*
1992		103.9	Table A.4 in Anonymous 1997*a*
1993		112.9	Table A.4 in Anonymous 1997*a*
1994		82.7	Table A.4 in Anonymous 1997*a*
1995		58.2	Table A.4 in Anonymous 1997*a*
1996		39.6	Table A.4 in Anonymous 1997*a*

Note: Values for the Lofoten survey (1985–1989) were corrected for changed target strength – length relationships according to the method described by Aglen and Nakken (1994).

fecundity (Kjesbu et al. 1998) suggests that at the stock level, recruitment will be influenced by the quality of spawners as well as their quantity.

At this point, an important distinction should be made between indices that apply to the stock as a whole and indices that apply to only a subset of the stock. Unlike liver weight, which can be measured for every cod, fecundity can only be measured for advanced vitellogenic females. In poor-condition years, the maturation process in cod may be modulated by atresia (Kjesbu et al. 1991) or perhaps skipped altogether (Oganesyan 1993; Burton et al. 1997). In the latter case, affected females will not be represented in the fecundity sample. Assuming that fecundity data collected from a sample of ripening females describe the reproductive potential of "all" females will give a misleading picture of the stock when cod are in poor condition and a percentage of females are atretic or skip spawning. For example, the difference in χ for an 85.5-cm cod in a poor-condition year (1988) and a better condition year (1989) was minimal (Fig. 3). However, this conclusion only applies to females that have accumulated sufficient energetic reserves to ripen and therefore be sampled for fecundity. Estimates of E should reflect the full range of reproductive responses to interannual variation in condition (e.g., changes in χ, atresia, skipped spawning) in proportion to their occurrence in the stock as a whole. Accurately describing the maturity composition of the stock is therefore a critical element of assessing its reproductive potential.

Calibrating year-specific fecundity–length relationships

To estimate E, year-specific fecundity–length relationships for 1985–1996 were developed as follows. Regression relationships were determined for each of the five years for which fecundity was measured (1986–1989 and 1991) using data in which both the independent and dependent variables had been transformed to natural logarithms (Table 5). Analysis of covariance indicated that both the slopes and intercepts of the relationships for 1986–1989 and 1991 varied significantly ($p < 0.01$) with condition as represented by the $\overline{\text{HSI}}_{\text{Jan.–Dec.},t-1}$ value for 61- to 70-cm cod ($r^2 = 0.91$, $n = 311$). Condition was therefore used to calibrate the coefficients of the year-specific fecundity–length relationships. The correlation between $\overline{\text{HSI}}_{\text{Jan.–Dec.},t-1}$ and the intercept of the fecundity–length relationship was statistically significant ($r^2 = 0.70$, $p < 0.01$, $n = 5$), as was the correlation between $\overline{\text{HSI}}_{\text{Jan.–Dec.},t-1}$ and the slope ($r^2 = 0.65$, $p < 0.01$, $n = 5$). Consequently, the correlations were used to calibrate the slopes and intercepts of the year-specific fecundity–length relationships for 1985–1996, including the five years for which observed values were available.

Estimating the number of mature females in the stock

Estimating the total number of cod by length

At the time of the Barents Sea survey, a portion of ripening cod have left the survey area for spawning grounds located off the coast of Norway, the most important being the Lofoten area. Consequently, neither the Barents Sea nor Lofoten survey samples the whole stock. To obtain a representative description of the whole stock, data from the two surveys were combined using the method described in detail in the Appendix. Briefly, the method partitions acoustic estimates of total abundance for each survey (Table 6) according to length, sex, maturity stage, and spawning experience (recruit versus repeat spawner) using information obtained from length-stratified subsampling of the trawl catch. The numbers of cod for both the Barents Sea and Lofoten surveys were then summed by category to estimate the total number of cod in each category for the stock. Thus, the acoustic estimates of total abundance determine the magnitude of the total number of cod by length whereas information from trawl sampling determines the demographic composition. This assumes that the number of cod outside the survey area is negligible and that cod were counted in only one of the two surveys. Cod in the near-bottom are not represented in the acoustic estimates of total abundance, and thus the accuracy of abundance estimates will be affected by spatial and temporal variation in the vertical distribution of cod (Aglen and Nakken 1997). It was not possible to quantify the degree to which these assumptions and biases affect the estimates of E. Wherever possible, estimates

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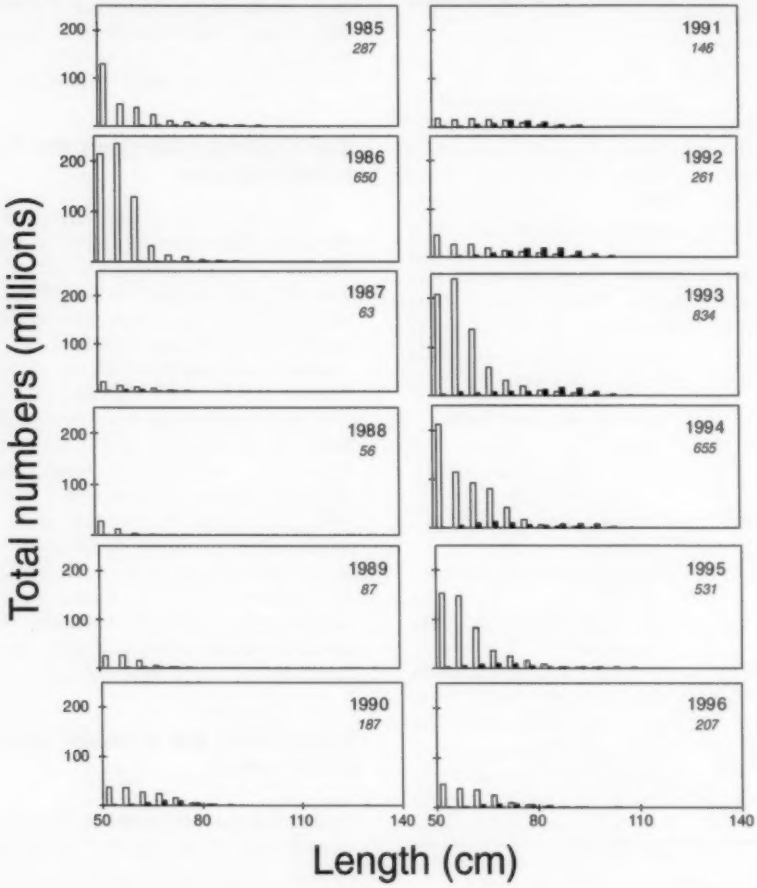
Fig. 4. Total numbers of NEA cod (females and males) by length (1985–1996). Open bars show data for the Barents Sea survey, and solid bars show data for the Lofoten survey. Totals across lengths for the two surveys combined are indicated in italics.



obtained by combining data from the two surveys were compared with data from other surveys (e.g., Russian acoustic/trawl surveys, egg surveys). The independence of the resulting estimates of the number of mature females from the VPA is discussed in the Appendix.

Interannual variation in the total number of cod by 5-cm length-class in the Barents Sea and Lofoten surveys (values include both females and males) illustrates how total abundance has varied for the length range corresponding to sexually mature cod (50–139 cm; Fig. 4). Cod in the aggregate length-classes 50–79, 80–109, and 110–139 cm are subsequently referred to as small, intermediate, and large cod, respectively. Small cod in the Barents Sea survey are numerically dominant. By comparison, the size composition of cod sampled in the Lofoten survey is shifted towards intermediate and large cod. The decrease in abundance of small cod in the Barents Sea that occurred between 1986 and 1987 is particularly striking. The cause of this sudden decrease is unknown. Comparing the time trend in cod abundance with that of liver condition indicates that when total abundance of cod was low (1987–1989), they were also in poor condition (Fig. 2A). Both factors acting in combination would reduce E.

Sex ratio by length

The percentage of females by 5-cm length-class is about 50% for small cod and then increases steadily such that about 100% of the large cod are female (Fig. 5). Increasing predominance of females with increasing length has been noted previously for this cod stock (Ponomarenko et al. 1980; Ponomarenko and Yaragina 1981) and for the Northern cod stock located off the coast of Newfoundland and Labrador (Ancellin 1954). Earlier maturation rates for males have also been noted in NEA cod (Ponomarenko and Yaragina 1981, 1994) as well as in other cod stocks (Fleming 1960; Oosthuizen and Daan 1974). Thus, sex ratios that become increasingly skewed towards females with increasing length could be interpreted as indirect evidence that differential growth results in differential maturation and (or) mortality rates. Size-dependent variation in sex ratios is an underappreciated demographic feature of exploited stocks. Stocks having a high proportion of large fish will have a buffer of eggs given the greater number of large, fecund females. Shifts in size composition towards small cod disproportionately reduce the number of females in the spawning stock. This, in combination with the lower χ of smaller cod (Fig. 3), makes it highly unlikely that

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Fig. 5. Percent females by length for combined Barents Sea and Lofoten survey data (1985–1996). Circles show data for the year indicated, and the solid line is a reference curve estimated by averaging values for 1993–1996.

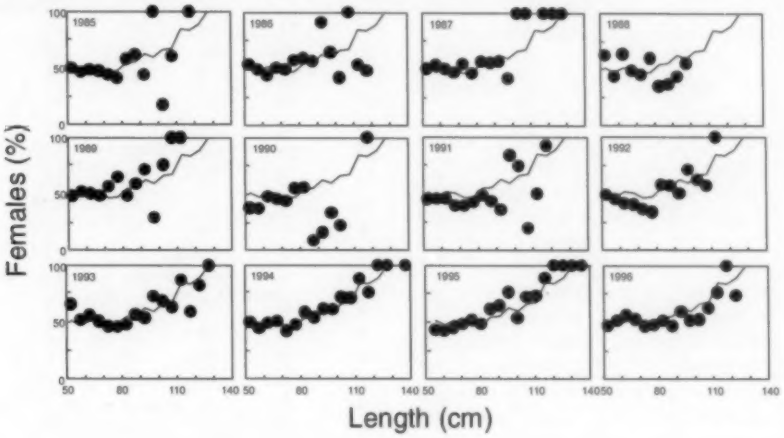
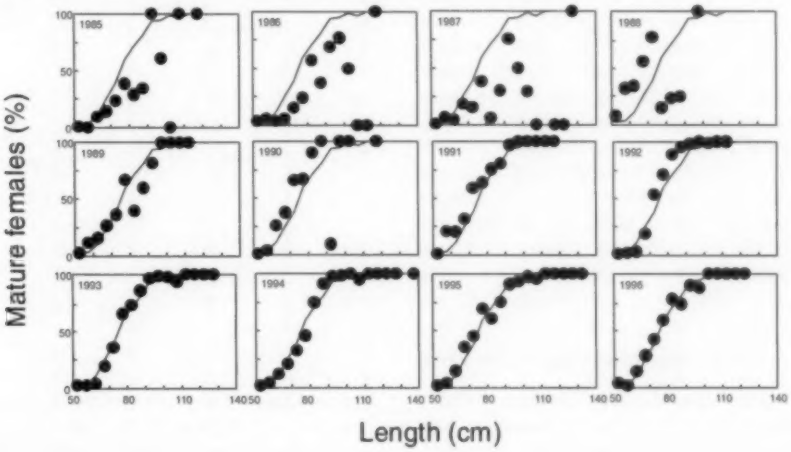


Fig. 6. Percent mature females by length for combined Barents Sea and Lofoten survey data (1985–1996). Circles show data for the year indicated, and the solid line is a reference curve estimated by averaging values for 1993–1996.



the assumption of a direct proportionality between E and SB, as expressed by eq. 1, is valid. Such factors could be part of the mechanistic basis of the positive correlation observed between age diversity and recruitment in Icelandic cod (Marteinsdottir and Thorarinsson 1998).

There is a second relevant feature in these data. In two of the years following the condition minimum in 1987 (1988 and 1990) the percentage of females in intermediate length-classes was frequently less than 50%. The bioenergetic costs of spawning are higher for female cod compared with males (Karlsen et al. 1995). Consequently, a reduced percentage of females in intermediate length-classes might reflect an increase in natural mortality of poor-condition females due to spawning. If true, then the anomalous values should propagate towards large cod (i.e., along cohorts) in subsequent surveys. Examination of the percentage of females by age for the Russian surveys did not indicate anomalously low percentages of females in October–December 1990 (N.A. Yaragina, unpublished data). Thus, inferring an increase in spawning mortality for females was not supported by independent data. Alternatively, intermediate-sized females may be underrepresented in the sampling if their maturation, migration, and (or) behaviour was affected by depleted energetic reserves.

Maturity composition by length

Maturity stages were assigned by visual assessment of gonads using either an eight-point scale (pre-1988) or a five-point scale (Table 2). Observations for cod classified as uncertain were omitted. Immature fish were those fish classified as belonging to either stage 0 (pre-1988) or stage 1 (1988 to the present). All other fish were considered to be mature.

Interannual variation in the percent mature females by 5-cm length-class (Fig. 6) suggests that in 1985–1987 the percentage of small mature cod was lower relative to the reference ogive estimated by averaging maturity-at-length values for 1993–1996. In 1986 and 1987 the percentage of mature females in several intermediate or large length-classes was equal or close to zero. This pattern is in sharp contrast with the

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Table 7. Number of observations for female NEA cod in each 5-cm length-class by year and survey.

Survey	Length (cm)	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996
Barents Sea	52.5	132	167	78	58	177	81	117	103	157	119	111	105
	57.5	65	220	74	35	216	73	101	79	152	105	132	106
	62.5	58	182	56	35	155	52	136	83	136	123	124	130
	67.5	54	76	46	17	100	50	131	61	92	117	121	113
	72.5	27	49	25	14	43	42	131	62	92	91	113	90
	77.5	13	29	9	10	25	23	99	62	75	72	92	82
	82.5	21	10	5	1	10	9	70	62	54	46	72	66
	87.5	8	10	3	2	15	1	40	40	48	34	46	41
	92.5	2	9	1	3	6	1	10	30	39	17	26	43
	97.5	1	5	3	1	2	0	5	13	22	15	8	27
	102.5	1	2	2	0	3	1	3	10	16	11	10	12
	107.5	0	1	0	0	2	0	2	1	6	7	4	6
	112.5	0	1	0	0	1	0	2	2	3	2	2	3
	117.5	1	1	0	0	0	0	0	0	1	1	2	0
	122.5	0	0	0	0	0	0	0	0	3	1	2	2
	127.5	0	0	0	0	0	0	0	0	1	1	0	0
	132.5	0	0	0	0	0	0	0	0	0	0	0	0
	137.5	0	0	0	0	0	0	0	0	0	0	0	0
Subtotal		383	762	302	176	755	333	847	608	897	762	865	826
Lofoten	52.5	0	4	10	24	2	0	0	3	11	2	5	0
	57.5	0	10	21	23	4	6	3	0	12	13	9	4
	62.5	4	11	35	29	9	15	3	1	9	30	19	21
	67.5	2	4	11	36	5	29	9	5	12	42	19	18
	72.5	4	5	9	31	6	28	15	7	22	35	29	31
	77.5	2	6	1	9	6	19	20	15	23	23	26	28
	82.5	4	11	4	4	4	7	12	33	39	32	21	26
	87.5	2	6	4	1	4	2	19	29	46	35	16	17
	92.5	2	6	1	0	1	1	10	34	57	54	33	17
	97.5	6	2	0	2	0	2	6	27	61	51	17	11
	102.5	0	1	1	0	0	0	3	19	37	45	23	10
	107.5	1	0	1	1	0	0	0	4	13	25	13	7
	112.5	0	0	0	0	0	0	0	3	7	17	8	2
	117.5	0	0	1	0	0	1	3	0	3	6	8	6
	122.5	0	0	1	0	0	0	0	0	4	2	5	2
	127.5	0	0	1	0	0	0	0	0	1	2	2	0
	132.5	0	0	0	0	0	0	0	1	0	0	2	0
	137.5	0	0	0	0	0	0	0	0	0	1	0	0
Subtotal		27	66	101	160	41	110	103	181	357	415	255	200
Total		410	828	403	336	796	443	950	789	1254	1177	1120	1026

Note: Subtotals for the Barents Sea and Lofoten surveys are given separately from the combined total at the bottom. Length indicates the midpoint of 5-cm length-classes.

sigmoidal model that is traditionally assumed to describe the size-dependent maturation dynamics of fish and that was observed from 1989 to 1996. During periods of low abundance, there is a concomitant reduction in the number of observations used to construct the ogives (Table 7), which will increase the noise, particularly for the large length-classes. However, there is independent evidence to support the interpretation of skipped spawning seasons. The affected length-classes correspond to repeat rather than recruit spawners (see data given below). Laboratory studies have shown that, in comparison with recruit spawners, repeat spawners experience more severe depletion of body reserves following spawning (Smith et al. 1990; Kjesbu et al. 1991) and are therefore more sensitive to variations in feeding regime (Kjesbu et al. 1991; Kjesbu and Holm 1994). Thus, in poor-condition years, repeat spawners might be unable to replenish energetic reserves depleted in the previous spawning season. This could result in atresia and (or) skipped spawning seasons. Skipped spawning seasons have been suggested previously for Northern cod (Morgan and Brattey 1996) and observed in food-limited cod held in captivity (Burton et al. 1997). Also, in 1987, no cod larger than 90 cm were caught in the fecundity samples collected off Vesterålen (Table 5). This is indirect evidence of the anomalous reproduction that year, since they were present in the stock (Table 7), albeit in reduced numbers. From a population dynamics perspective, the reproductive potential of cod in intermediate length-classes is of critical importance because a higher percentage are mature compared with small cod and, unlike large cod, they can be numerically abundant enough to make a substantial contribution to *E.*

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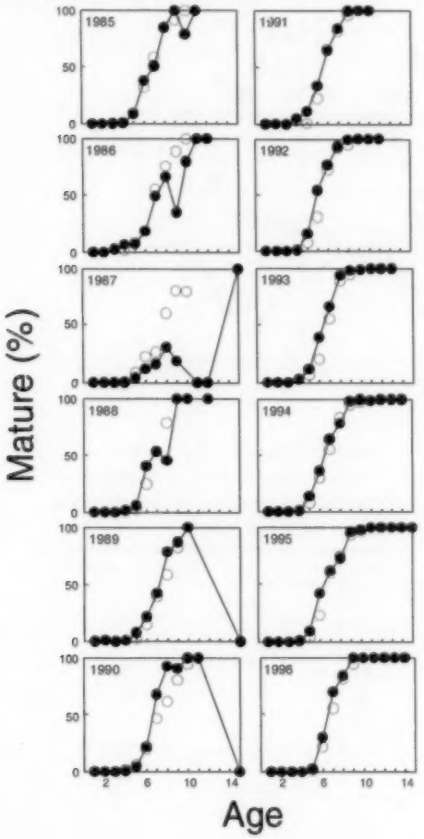
Fig. 7. Percent mature by age for Russian (open circles, males and females combined) and Norwegian (solid circles connected by solid line, females only) surveys (1985–1996).



Maturity ogives for the combined Barents Sea and Lofoten surveys were compared with the ogives estimated using Russian data from the acoustic/trawl survey conducted in October–December of the preceding year and from sampling of the commercial catch (table 3.5 in Anonymous 1997*a*). In this case, the proportion of mature females is estimated by age rather than by length (Fig. 7). Differences between the two surveys are expected because (*i*) the Russian ogives were constructed using data for both males and females whereas the Norwegian ogives apply only to females and (*ii*) differences between the two countries existed in age estimates for 1990 and 1991 (internal report, Institute of Marine Research/Polar Research Institute of Marine Fisheries and Oceanography, June 30, 1995). Despite these differences, agreement between the two ogives for recent years (1991–1996) was good. During the poor-condition years (1986–1988), the Norwegian ogives indicate markedly lower values for some age-classes. This is most noticeable for 1987 when high percentages of the cod between the ages of 6 and 12 were classified as immature (i.e., nonspawning) in the Norwegian surveys. As data used to construct the Russian ogives describe the maturity stage of cod 2–4 months before the Norwegian surveys, the decrease in the percent mature might indicate that atresia occurred between the two surveys. If this reduction in the number of spawning females is real, then the VPA-based SB, which is estimated using the Russian ogives, overestimated the reproductive potential of the stock in the poor-condition years.

Spawning experience by length

Age at first maturity was determined from a characteristic otolith pattern first described by Rollefsen (1933). Spawning experience was determined from the difference between the age at capture and the age at first maturity. For recruit spawners the difference is zero whereas the difference is 1 or greater for repeat spawners.

As expected, the percentage of mature repeat-spawning females by 5-cm length-class increases with increasing age such that all large cod are repeat spawners (Fig. 8). However, a surprisingly high percentage of small cod were classified as repeat spawners in 1988–1990 and 1995–1996. Given that the majority (>80%) of fish in these size-classes are immature (Fig. 7), it seems highly unlikely that the majority of mature individuals are actually repeat spawners. Both time periods are associated with low values of $HSI_{Jan.-Dec.}$ (Fig. 2A). It is therefore possible that the pattern in the otoliths that is interpreted as indicating the onset of maturity can also be generated by a reduction in growth rates. If so, then poor growth during the immature stage will be confounded with the onset of maturation. Studies that have used the age at first maturity for constructing maturity ogives for NEA cod over the historical (presurvey) time period (e.g., Jørgensen 1990; Beverton et al. 1994) may require reinterpretation. This result deserves further investigation, perhaps using the otolith database for the historical time period. Given the ambiguity of the otolith information, arithmetic means of the percent repeat spawners by length for a relatively stable time period (1992–1995) were used to partition *E* into total egg production by repeat and recruit spawners (Table 8).

Interannual variation in *E*

The number of mature female cod in each 5-cm length-class was estimated by summing the number of cod in the Barents Sea and Lofoten surveys (Fig. 4) within each length-class and then multiplying that value by the proportion of females (Fig. 5) and the proportion of mature females (Fig. 6) for that length-class. Egg production by length was estimated by multiplying the number of mature female cod in each length-class by the fecundity estimated for the midpoint of that length-class using the year-specific fecundity–length relationships described above.

Egg production by 5-cm length-class shows considerable interannual variation in the mode and length range spanned (Fig. 9). In 1987 and 1988, when condition and abundance were low, egg production was negligible relative to other years. A pulse of egg production appeared in 1990 (mode at 72.5 cm) due to recruitment of the strong 1983 year-class to the spawning stock. In following years, this pulse propogated through to increasing lengths, gradually decaying and disappearing by 1996. A secondary pulse appeared in 1994 (mode at 67.5 cm); however, this pulse decayed more rapidly (≈3 years) compared with the pulse generated by the 1983 year-class (≈6 years). These data clearly illustrate the importance of the intermediate length-classes to the reproductive potential

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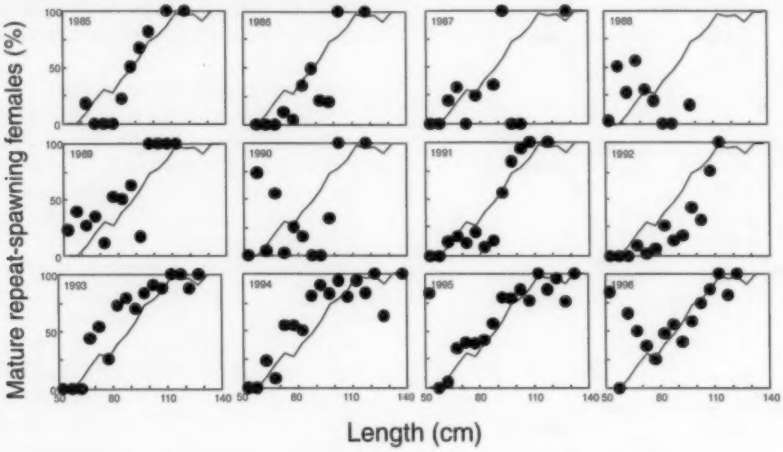
Fig. 8. Percent mature, repeat-spawning females by length for combined Barents Sea and Lofoten survey data (1985–1996). The standard curves obtained by averaging data for 1992–1995 are shown as solid lines.



of the stock. As noted earlier, cod in intermediate length-classes have the combined attributes of higher numerical abundance (relative to large cod) and higher fecundity and proportions mature (relative to small cod).

Estimates of E were obtained by summing egg production across length. Conceptually, they correspond to an estimate of the potential total egg production (E_{pot}) because the fecundity estimates used represent the total number of vitellogenic oocytes in the prespawning ovary. As an independent check on the accuracy of the estimation method, E_{pot} for 1985 was compared with an estimate of total egg abundance from egg surveys in the Lofoten area in the same year (Sundby and Bratland 1987). The latter represents a spatially integrated measure of the abundance of eggs in stage 1 (0–2 days), stage 2 (2–5 days), and stage 3 (5–7 days). The egg survey therefore corresponds more closely to the realized total egg production (E_{real}), i.e., E_{pot} modified by atresia and (or) mortality. In 1985, E_{pot} was estimated to be 40×10^{12} compared with an egg survey estimate of E_{real} of 21×10^{12}. Given the differences in the data used to generate these two estimates, it is gratifying that they are in the same order of magnitude. Furthermore, the difference between E_{pot} and E_{real} ($E_{real}/E_{pot} = 0.53$) was within expected values, given that laboratory studies have shown that the cumulative egg mortality in the first few days after fertilization is on the order of 50% (Nissling and Westin 1991; Solemdal et al. 1995).

Table 8. Standard values used to partition number of mature female NEA cod by length into the number of mature, repeat-spawning females.

Length (cm)	Repeat spawners (%)
52.5	0
57.5	0
62.5	9
67.5	20
72.5	30
77.5	27
82.5	39
87.5	47
92.5	58
97.5	73
102.5	78
107.5	86
112.5	98
117.5	96
122.5	97
127.5	91
132.5	100
137.5	100

Note: Percentages were calculated by averaging values for 1992–1995 for each 5-cm length-class. Length indicates the midpoint of 5-cm length-classes.

Relationship between *E* and VPA-based SB

The main objective of this study was to test the assumption that E is directly proportional to VPA-based SB. Direct proportionality implies that (*i*) the relationship is linear, and therefore the number of eggs produced per unit spawner biomass (E/SB) is constant and equal to the slope of the relationship and (*ii*) when SB is equal to zero, then E will also be zero. In the discussion that follows, it is convenient to use a reference point of 500 000 metric tons (t), which is the level currently regarded by management as the minimum biologically acceptable level (MBAL; Jakobsen 1993) of SB.

For NEA cod the relationship between E and VPA-based SB deviates from the assumption of direct proportionality in several respects (Fig. 10). Above the MBAL, E was twice as variable (min./max. = 0.33) as the VPA-based SB (min./max. = 0.67). The variation in E observed for years when the VPA-based SB was higher than the MBAL (1991–1995) can be attributed to a rapid shift in the size composition of spawning females from 1991 to 1995 (Fig. 11). During this time period the mode of the size distribution increased by 15 cm from 72.5 cm in 1991 to a maximum of 87.5 cm in 1993 and then decreased by 20 cm to 67.5 cm in 1995. The disproportionately higher E in years where spawners are larger can be

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Fig. 9. Egg production by length for combined Barents Sea and Lofoten survey data (1985–1996). Totals across lengths are indicated in italics.

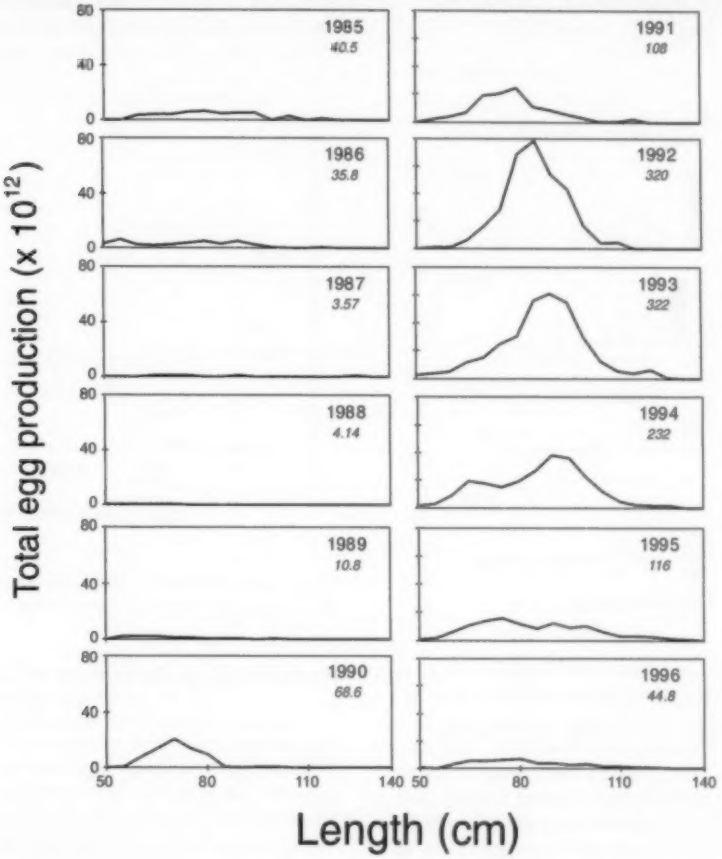
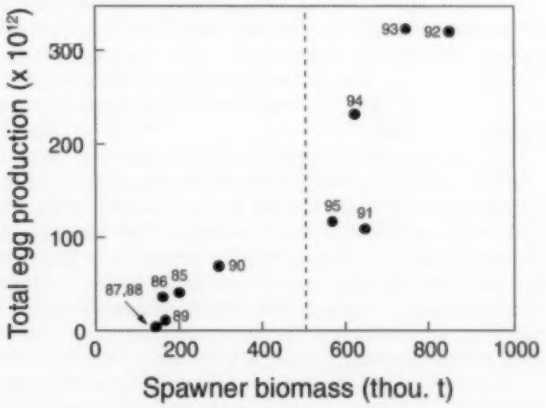


Fig. 10. Relationship between VPA-based SB and total egg production for NEA cod. Data labels correspond to year. The vertical broken line indicates the minimum biologically acceptable level of 500 000 t (Jakobsen 1993).



attributed to the multiplicative effects of larger cod having a higher χ (Fig. 3), a higher proportion of females (Fig. 5), and a higher percentage of mature females (Fig. 6).

Ricker (1975) proposed that a basic property of the spawner–recruit relationship was that it passed through the origin. This property is so obvious that it has rarely, if ever, been closely examined. For NEA cod, E was extremely low in 1987 and 1988 when the VPA-based SB were about 143 000 and 145 500 t, respectively (Fig. 10). Recruitment indices for the 1987 and 1988 year-classes were also low (see below). Assuming that the relationship between E and SB is linear and passes through the origin potentially overestimates the reproductive potential when abundance is low.

The relationship between E and VPA-based SB near the origin was examined in greater detail by including estimates of E from egg surveys of the the North Sea and the Irish Sea (Table 1). Plotted on the same scale, the data suggest that the relationship between E and VPA-based SB for cod stocks in general is nonlinear, with the variance in E increasing with increasing SB (Fig. 12). Pooling data for stocks differing in size to the degree of the Irish Sea and NEA cod stocks mimics the changes that can occur in a collapsing cod stock. For example, the VPA-based SB of Northern cod decreased from 1.6×10^6 t in 1962 to 22 000 t in 1992 (Bishop et al. 1993). Thus, Northern cod can be presumed to have undergone a nonlinear decrease in E similar to that described for cod stocks generally in Fig. 12. Unfortunately, the magnitude of the decrease will never be known with precision owing to the paucity

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Fig. 11. Length distribution of mature females for 1991–1995. Data are for the combined Barents Sea and Lofoten survey data. The solid bar indicates the mode of the length distribution.

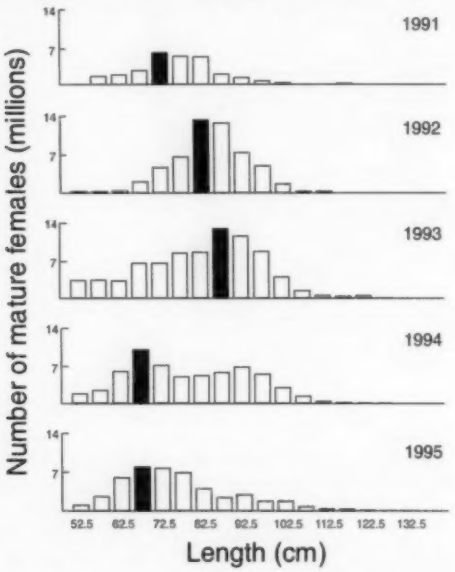
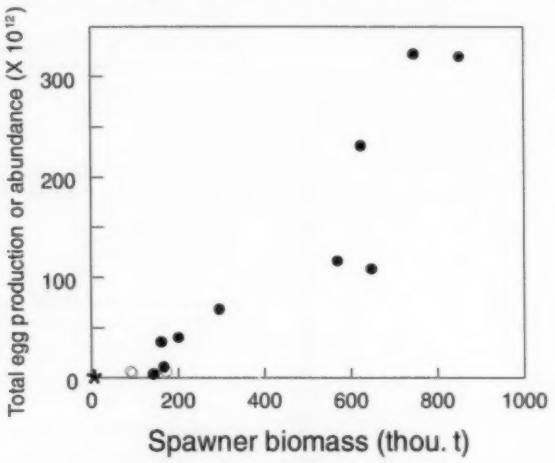


Fig. 12. Relationship between VPA-based SB and total egg production for NEA cod (solid circles, 1985–1996) and field-based estimates of total egg abundance for North Sea cod (open circles, 1976 and 1987–1988) and Irish Sea cod (asterisk, 1995).



of detailed fecundity information that could be used to calculate E. Hutchings and Myers (1994) have suggested that decreases in E were a factor contributing to the long-term demographic collapse of Northern cod. However, their estimates of E were calculated using the fecundity–age relationship given in May (1967) and do not account for the poor physiological condition of two of the three geographical subcomponents of the Northern cod stock that was observed in the early 1990s (Lilly 1997). This decrease in condition likely amplified the decline in reproductive potential of the stock.

Our conclusion that the assumption of proportionality between E and VPA-based SB is invalid for NEA cod stocks needs to be confirmed in other stocks. Estimates of total abundance that are independent of the VPA are essential, particularly if it is uncertain whether VPA results are an accurate description of both the magnitude and rate of change in abundance of mature females. Unfortunately, few stocks have the advantage of acoustic/trawl surveys that can empirically estimate stock abundance and circumvent some of the problems associated with VPA estimates of abundance. There is also a need for basic fecundity data and quantitative descriptions of how this important life history term varies interannually. The data required to assess the maturity composition of a stock are often lacking because of inappropriately timed groundfish surveys (Trippel et al. 1997*b*). Developing these databases should be a goal of future recruitment research because the pattern of variability shown in the relationship between E and VPA-based SB represents the "ground zero" (i.e., $t = 0$) that will subsequently be modified by mortality processes to yield the overly familiar scattershot pattern of spawner–recruit relationships for cod (fig. 5*a* in Jakobsen 1996 illustrates the variation observed when data for several cod stocks are pooled). If it can be confirmed for other stocks and (or) species that the relationship between VPA-based SB and E consistently deviates from direct proportionality, then the continued use of VPA-based SB as a proxy for reproductive potential is not justified.

Relationship between recruitment and *E*

A primary goal of fisheries science is to identify the factors responsible for generating interannual variation in recruitment. If VPA-based SB is an imprecise and biased measure of the reproductive potential of fish stocks, will estimates of E increase our ability to explain recruitment variation? To address this question, the relationship between recruitment and VPA-based SB was contrasted with the relationship between recruitment and E. Given the preceding criticism of the VPA results, acoustic estimates of the abundance at age 1 in the Barents Sea survey in year $t + 1$ (table A2 in Anonymous 1997*a*) were used as the recruitment index for the year t. Excluding the observation for 1986 (the 1985 year-class), the acoustic estimates of the abundance at age 1 are strongly correlated with the VPA estimates ($r^2 = 0.96$, $p < 0.0001$, $n = 10$). A discrepancy between the VPA and survey-based estimates of the strength of the 1985 year-class has also been noted for the 0-group survey (Sundby et al. 1989).

There are several important differences between the relationships for E and VPA-based SB. Firstly, unlike the relationship between VPA-based SB and recruitment (Fig. 13A), the relationship between E and recruitment has the conceptually appealing property of approaching the origin (Fig. 13B). Secondly, the variation observed in E (min./max. = 0.001) is closer to the magnitude of variation observed in recruitment to age 1 (min./max. = 0.00075) and considerably higher than the magnitude of variation observed in the VPA-based estimate of SB (min./max. = 0.17). Lastly, recruitment was highest for intermediate levels of E, which suggests a form of compensatory recruitment regulation. Cannibalism is an important compensatory mechanism in cod stocks (Bogstad et al. 1994). The importance of considering how variation in both E and cannibalism influences recruitment is illustrated by the 1990 year-

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Fig. 13. Relationship between (A) VPA-based SB in year t and the acoustic estimate of the total abundance of age 1 cod in year $t + 1$ and between (B) E in year t and the acoustic estimate of the total abundance of age 1 cod in year $t + 1$.

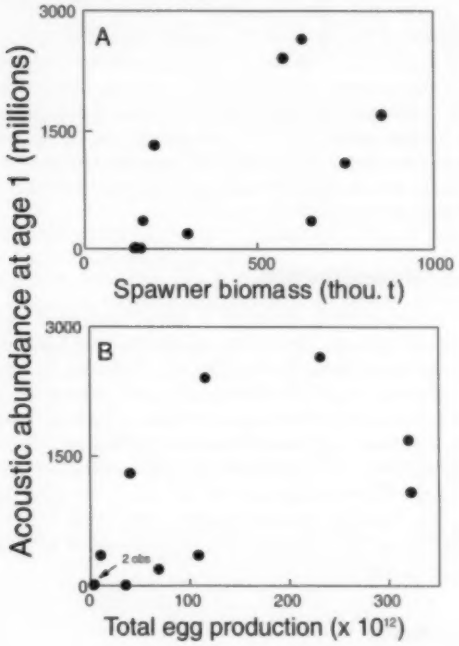
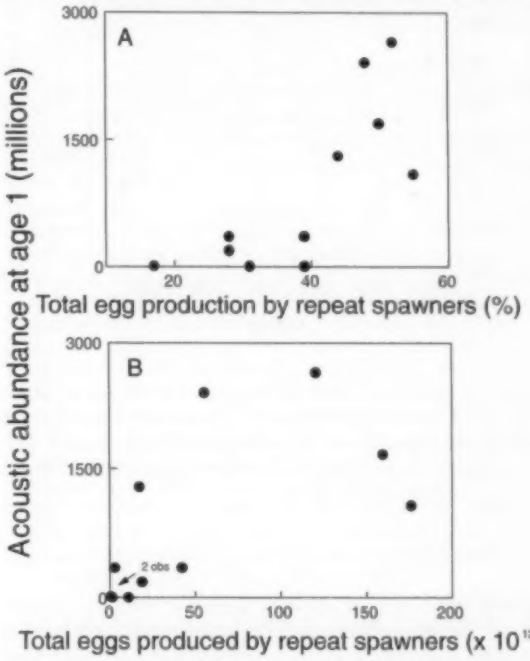


Fig. 14. Relationship between (A) the percentage of total eggs produced by repeat spawners in year t and the acoustic estimate of the total abundance of age 1 cod in year $t + 1$ and between (B) the total number of eggs produced by repeat spawners in year t and the acoustic estimate of the total abundance of age 1 cod in year $t + 1$.



class. An intermediate value of E was observed in 1990 (Fig. 9). However, the 1990 year-class was produced when stock abundance was just beginning to increase (Fig. 4) and cannibalism rates were about 1/10 of those than in subsequent years (table A16 in Anonymous 1997*a*). This may have provided a window of opportunity for the 1990 year-class to develop into one that is presently dominating the fishery (Anonymous 1997*a*).

The quantity of eggs produced is not the only factor to consider when evaluating the reproductive potential of the stock. There is evidence that egg quality (e.g., survivorship, egg size, buoyancy, hatching success) varies according to maternal size (Kjesbu et al. 1992; Solemdal et al. 1993) and spawning experience (Solemdal et al. 1995; Kjesbu et al. 1996; Trippel et al. 1997*a*). Both are traits that reflect the growth history of mature cod. To assess how variation in egg quality influences recruitment, E was partitioned into production by recruit spawners (E_{rec}) and the production by repeat spawners (E_{rep}) using the standard values given in Table 8. Recruitment was low in years when the percentage of eggs produced by repeat spawners ($E_{rep}/(E_{rec} + E_{rep}) \times 100$) was low (Fig. 14A). The relationship between recruitment and E_{rep} is also suggestive of a dome-shaped relationship (Fig. 14B). Contrasting Fig. 14B with Fig. 13A suggests that expressing the recruitment potential of a stock in terms of both the quantity and quality of eggs produced may help to resolve the longstanding debate concerning whether or not there is a functional relationship between spawners and recruits. The same argument was put forward for North Sea haddock (*Melanogrammus aeglefinus*) by Hislop (1988) who advocated downweighting the egg contribution of "precocious" 2-year-old haddock because they produce significantly smaller eggs which are presumably of inferior quality.

Implications for management of NEA cod

Presently, the management protocol for NEA cod involves generating short-term projections of VPA-based SB and using these values to recommend harvesting rates that are consistent with target levels of fishing mortality. No consideration is given to variation in reproductive potential beyond the standard assumption that the VPA-based SB is proportional to E. The preceding analysis strongly suggests that this approach is insensitive to the true variation in reproductive potential of the stock. Therefore, survey-based indices of total abundance and reproductive potential should be incorporated into the analytical assessment of stock status to indicate the quantity and quality of spawners. Greater use of survey-based abundance indices in stock assessment has also been recommended in the wake of the collapse of the Northern cod stock (Walters and Maguire 1996).

Several possible survey-based indices could be used to describe the reproductive potential of NEA cod. For example, reproductive potential could be expressed as the relative fecundity of the stock (χ_{stock}). This index could be estimated by multiplying the number of mature females by length by the weight-at-length for the midpoint of the length-class (estimated using the regressions in Table 3) to obtain the total

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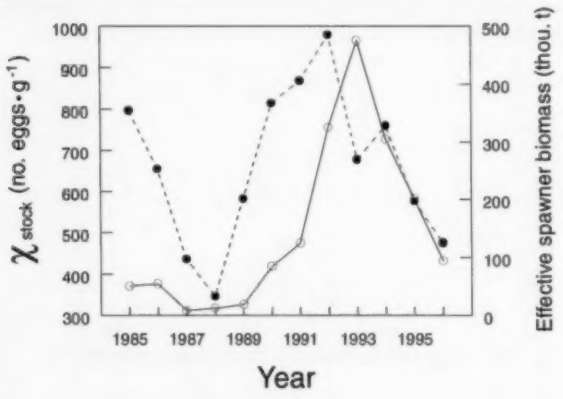
Fig. 15. Time series of the relative fecundity (χ_{stock}, solid circles) and effective SB (open circles) of NEA cod stock as described in the text.



biomass of spawning females (SB_{fem}). This value is not independent of E, as the only difference between them results from replacing the fecundity-at-length of prespawning females with their weight-at-length, parameters that covary as noted above. The χ_{stock} is then calculated as

$$\chi_{stock} = \frac{E}{SB_{fem}}. \quad (3)$$

The time trend in χ_{stock} shows a threefold level of variation (Fig. 15), further evidence that the proportionality assumption is invalid. Although it might seem preferable to have high spawner abundance and high χ_{stock} (e.g., 1992), this situation has the disadvantage that cannibalism rates will be high. The combination of high χ_{stock} and intermediate spawner abundance, and hence lower cannibalism rates, might be more effective in generating strong year-classes (e.g., 1990).

Conclusion

It has often been noted that the magnitude of variation in SB is insufficient to account for the variation observed in recruitment to marine fish stocks (Shepherd et al. 1984; Wooster and Bailey 1989; Rijnsdorp et al. 1991). Consequently, ecological and (or) environmental factors affecting prerecruit mortality have been invoked as the primary determinants of recruitment variation. This study has shown that the magnitude of variation in survey-based estimates of E is greater than that observed in VPA-based SB and much closer to the level of variation in recruitment. Process-oriented models of the recruitment dynamics of marine fish require integrating the production processes affecting E (growth, reproduction) with mortality processes affecting larval and juvenile survival (Ulltang 1996). Decades of research into the early life history of marine fish can be used to quantify interannual variation in prerecruit mortality. The results presented here strongly suggest that similar consideration should be given to estimating E independently of the VPA wherever possible. Interactions between production and mortality processes are likely, given that stocks with a high proportion of large fish potentially produce eggs over a longer period of time, thereby increasing not only the number of eggs but also their probability of survival (Lambert 1990; Kjesbu et al. 1996).

In closing, we hope to have demonstrated that resolving the complex nature of the relationship between SB and recruitment is more than just a matter of cataloging the statistical deficiencies of the data and (or) models used. The relationship must be placed firmly in the context of the underlying biological, ecological, and environmental processes governing the growth, reproduction, and mortality of fish. There is no substitute for the collection, in the laboratory and in the field, of detailed information describing the reproductive potential of individuals and stocks. The data are hard-won but the insights to be gleaned potentially great.

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Appendix

Data were analyzed using a modified version of a SAS program written by K. Korsbrekke (Institute of Marine Research, Bergen). Only data from the Barents Sea and Lofoten surveys were included in the analysis. There have been several

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changes in survey methodology which are documented in Jakobsen et al. (1997). In some cases the changes have been accounted for in the acoustic estimates of total abundance (e.g., change from bobbins to rock-hopper gear, change in target strength). Changes in spatial coverage have not been accounted for; however, these changes will have a greater effect on data pertaining to age 1 and 2 cod compared with older cod (Jakobsen et al. 1997). Given the potential for biases that result from changes in survey methodology to influence our results, we have emphasized comparing our data with data that were collected independently (e.g., Russian acoustic/trawl surveys, synoptic-scale egg surveys).

For each survey, the acoustic estimate of total abundance (N; Table 6) was partitioned into 5-cm length-classes as follows:

$$\text{(A1)}\quad N_l = \frac{n_l}{\sum_l n_l} \times N$$

where N_l is the total number by length and n_l is the total number of cod of length l captured during trawl sampling (i.e., the sum of the number of cod of length l caught at each station corrected for differences among stations in tow distance, gear type, and subsampling of large catches). Following Anonymous (1994), the number of cod in each length, sex, maturity, and spawning experience category ($N_{l,s,m,r}$) was estimated by

$$\text{(A2)}\quad N_{l,s,m,r} = N_l \times \frac{k_{l,s,m,r}}{k_l}$$

where $k_{l,s,m,r}$ is the number of fish of length l, sex s, maturity m, and spawning experience r that were subsampled for subsequent age determinations and k_l is the number of fish of length l (i.e., $k_l = \Sigma_s \Sigma_m \Sigma_r k_{l,s,m,r}$). Values of $k_{l,s,m,r}$ were corrected for the length-stratified subsampling of the catch for age determinations (Morgan and Hoenig 1993). As an indication of variability in sampling effort, the numbers of females that were sampled in each 5-cm length-class for maturity and otoliths (i.e., $k_{l,\text{fem}} = \Sigma_m \Sigma_r k_{l,s,m,r}$) are given for the Barents Sea and Lofoten surveys in Table 7.

The total number of cod of length l in the stock ($N_{l,\text{stock}}$) was estimated as

$$\text{(A3)}\quad N_{l,\text{stock}} = N_{l,\text{Bar}} + N_{l,\text{Lof}}$$

where $N_{l,\text{Bar}}$ and $N_{l,\text{Lof}}$ are the total number of cod of length l in the Barents Sea and Lofoten surveys, respectively (from eq. 4). Thus:

$$\text{(A4)}\quad \sum_l N_{l,\text{stock}} = N_{\text{Bar}} + N_{\text{Lof}}$$

where N_{Bar} and N_{Lof} are the acoustic estimates of the total number of cod in the Barents Sea and Lofoten surveys, respectively (from Table 7).

Values of $N_{l,\text{Bar}}$ and $N_{l,\text{Lof}}$ could be progressively subdivided according to sex, maturity, and spawning experience. The total number of cod in the stock belonging to each category ($N_{l,s,m,r,\text{stock}}$) was estimated by

$$\text{(A5)}\quad N_{l,s,m,r,\text{stock}} = N_{l,s,m,r,\text{Bar}} + N_{l,s,m,r,\text{Lof}}$$

For example, the number of females by length in the stock was estimated by

$$\text{(A6)}\quad N_{l,\text{fem,stock}} = N_{l,\text{fem,Bar}} + N_{l,\text{fem,Lof}}$$

Thus, the percentage of females by length in the stock by length ($P_{l,\text{fem,stock}}$) was estimated by

$$\text{(A7)}\quad P_{l,\text{fem,stock}} = \frac{N_{l,\text{fem,Bar}} + N_{l,\text{fem,Lof}}}{N_{l,\text{Bar}} + N_{l,\text{Lof}}} \times 100\,(\%)$$

The percentage of mature females by length in the stock ($P_{l,\text{fem,mat,stock}}$) was estimated by

$$\text{(A8)}\quad P_{l,\text{fem,mat,stock}} = \frac{N_{l,\text{fem,mat,Bar}} + N_{l,\text{fem,mat,Lof}}}{N_{l,\text{fem,Bar}} + N_{l,\text{fem,Lof}}} \times 100\,(\%)$$

Similarily, the percentage of mature, repeat-spawning females by length in the stock ($P_{l,\text{fem,mat,rep,stock}}$) was estimated by

$$\text{(A9)}\quad P_{l,\text{fem,mat,rep,stock}} = \frac{N_{l,\text{fem,mat,rep,Bar}} + N_{l,\text{fem,mat,rep,Lof}}}{N_{l,\text{fem,mat,Bar}} + N_{l,\text{fem,mat,Lof}}} \times 100\,(\%)$$

Estimates of $N_{l,\text{stock}}$ can be considered independent from the VPA estimates of total abundance in the following respects: (*i*) they are based solely on survey data and are independent of the commercial catch, (*ii*) they make no assumptions about the magnitude of both natural and fishing mortality, (*iii*) acoustic estimates of the total abundance-at-age (N in Table 6 partitioned by age rather than by length) for the Barents Sea and the Lofoten surveys were only two of eight tuning indices used in the assessment (Anonymous 1997*a*) (the other tuning indices used include data from trawl and acoustic sampling during Russian surveys, Norwegian sampling of commercial catch, Russian sampling of commercial catch, Norwegian trawl sampling of the Svalbard region, and Norwegian trawl sampling of Barents Sea; in practice, the acoustic indices are given greater weight for tuning prerecruit age-classes whereas the commercial indices have greater weight for tuning the recruited age-classes (B. Bogstad, Institute of Marine Research, personal communication)), (*iv*) trawl information from the Lofoten survey was not used in tuning, (*v*) for the Lofoten surveys, only acoustic data from 1989 to 1995 were used in tuning, and (*vi*) in the present study, data for the Barents Sea and Lofoten surveys were combined to represent the whole stock whereas the tuning procedures consider the two surveys separately.

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DISCUSSION

Comment: Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams[1]

Roger G. Young and Alexander D. Huryn

Marzolf et al. (1994) describe an elegant method for measuring ecosystem metabolism in small streams that is now in wide use. The equation they provide for calculating the reaeration flux of oxygen through the water's surface, however, appears to be incorrect. Here we show why we believe this equation is incorrect and propose an alternative and more versatile equation that we have tested in a number of small streams under a variety of conditions. We also emphasize the importance of using the mean oxygen deficit to determine reaeration flux, particularly in streams that have substantial temperature changes.

The equation in question (i.e., Marzolf et al. 1994, p. 1593, eq. 2) is

(1) $$\text{Reaeration flux} = DO_{deficit} \times e^{-k_{oxygen} \times T}$$

where $DO_{deficit}$ ($mg \cdot L^{-1}$) is the difference between the concentration of dissolved oxygen at 100% saturation and the observed concentration, k_{oxygen} (min^{-1}) is the reaeration coefficient determined using injections of propane to simulate oxygen dynamics, and T (min) is the travel time along a specified reach. The negative exponential form of the equation is problematical. Consider as a simple example a stream with a reaeration coefficient (k_{oxygen}) of $0.07 \cdot min^{-1}$ and a fixed dissolved oxygen deficit ($DO_{deficit}$) of $+1.0\ mg \cdot L^{-1}$. According to eq. 1, oxygen flux from a parcel of water during 10 min of travel down the reach will be $0.497\ mg \cdot L^{-1}$ whereas flux from the same parcel of water during 60 min of travel will be $0.015\ mg \cdot L^{-1}$. In other words, given identical conditions and the form of eq. 1, short reaches will have larger fluxes of dissolved oxygen than long reaches (Fig. 1). Given the constant conditions imposed, this scenario is clearly erroneous.

Error in the calculation of the reaeration flux has important implications for estimates of ecosystem metabolism, because such estimates are sensitive to the accuracy of the reaeration flux. Use of eq. 1 may result in substantial negative gross metabolism during the day and positive net metabolism at night in streams with high reaeration coefficients and overestimation of metabolism in streams with low reaeration coefficients (R.G. Young and A.D. Huryn, personal observation).

To solve this problem, we advocate the use of the following equation to calculate the correct reaeration flux of oxygen along small stream reaches:

(2) $$\text{Reaeration flux} = DO_{deficit} \times k_{oxygen} \times T$$

This equation is similar to that used in traditional single-station and two-station analysis of metabolism where the reaeration coefficient (k_{oxygen}) is multiplied directly by the oxygen deficit ($DO_{deficit}$) to determine the reaeration flux of oxygen (Owens 1974; Hall and Moll 1975; Bott 1996). Multiplication by the travel time (T) allows the calculation of total oxygen flux ($mg \cdot L^{-1}$) along the reach, which then can be used to determine metabolism using further equations provided by Marzolf et al. (1994).

In streams without forested canopies, such as the grassland streams that we have studied in New Zealand, there can be substantial changes in temperature of a parcel of water as it flows through even relatively short reaches (e.g., 1–3°C during 60 min of travel). Such variation in temperature will result in substantial changes to the dissolved oxygen deficit during the period of transit (T). In addition to changes caused by the physical effect of temperature on oxygen solubility, losses and gains of oxygen by community metabolism may also alter the dissolved oxygen deficit during transit. Under situations where the dissolved oxygen deficit is expected to change substantially along the study reach, we advocate using the mean of the upstream and downstream dissolved oxygen deficits, or

(3) $$\text{Reaeration flux} = (DO_{deficit\ 1} + DO_{deficit\ 2})/2 \times k_{oxygen} \times T$$

where $DO_{deficit\ 1}$ and $DO_{deficit\ 2}$ are dissolved oxygen deficits estimated for a parcel of water as it passes the beginning and

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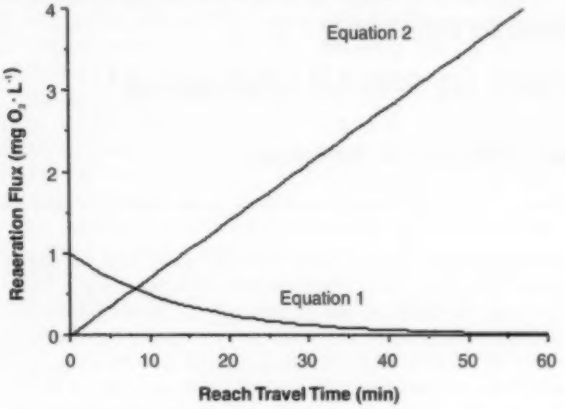
Fig. 1. Comparison of the relationship between reach travel time and reaeration flux calculated from either eq. 1 or eq. 2. For both equations we have used a constant reaeration coefficient (k_{oxygen}) of 0.07·min^{-1} and dissolved oxygen deficit ($DO_{deficit}$) of +1.0 mg·L^{-1}.



the end of the study reach. This equation assumes that the change in dissolved oxygen deficit is linear during transit. Given that factors that may underlie short-term shifts in the dissolved oxygen deficit are multiple and potentially independent (e.g., temperature, respiration, photosynthesis), this assumption appears to be reasonable.

Finally, we have also noticed an additional but very minor problem with fig. 2 in Marzolf et al. (1994). The line representing the upper boundary of community respiration (CR_{24}) should cross the *y*-axis where metabolism equals zero, not at –0.2 mg O_2·reach^{-1}·s^{-1} as shown.

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Reply: Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams[1]

Erich R. Marzolf, Patrick J. Mulholland, and Alan D. Steinman

Young and Huryn (1998) note that in our paper describing an oxygen mass balance approach for determining whole stream metabolism (Marzolf et al. 1994), there is an error in the equation for calculating the reaeration flux of oxygen over the study reach (eq. 2). They are correct. The equation as given in the original paper was

(1) Reaeration flux = $DO_{deficit} \times e^{-k_{oxygen} \times T}$

where $DO_{deficit}$ is the difference between the concentration of dissolved oxygen at 100% saturation and the observed concentration, k_{oxygen} is the reaeration coefficient for oxygen, and T is the average water travel time through the study reach. This equation is in error (as depicted in fig. 1 of Young and Huryn (1998)) and, in fact, was not the one we actually used in calculating reaeration flux. The equation used to calculate reaeration flux in the determinations of stream metabolism presented in this paper and in a recent paper (Mulholland et al. 1997) was

(2) Reaeration flux = $DO_{deficit} \times (1 - e^{-k_{oxygen} \times T})$

The erroneous drastic decline in reaeration with increasing travel time predicted by eq. 1 is not a problem in eq. 2. However, eq. 2 above has another limitation; it is not appropriate for situations in which multiple processes (e.g., reaeration and metabolism) are influencing the dissolved oxygen concentration in a parcel of water as it moves through the study reach. In many streams, respiration maintains the $DO_{deficit}$ at relatively constant levels in a water parcel as it moves downstream. In such streams, use of eq. 2 above substantially underestimates reaeration flux. The underestimation of reaeration flux is greatest in streams with relatively high values of k_{oxygen} and (or) relatively long average water travel times. For example, for the situation in which $k_{oxygen} \times T = 2$ (similar to values for the Walker Branch study reach on many dates), reaeration flux is underestimated by a factor of 2.3. Young and Huryn (1998) suggest that the following equation be used to compute reaeration flux:

(3) Reaeration flux = $DO_{average\ deficit} \times k_{oxygen} \times T$

where $DO_{average\ deficit}$ is the average $DO_{deficit}$ at the upstream and downstream ends of the study reach. We agree completely with this suggestion. We have recalculated metabolism values for Walker Branch using this formulation of the reaeration flux

Table 1. Revised metabolic data for the 62-m, first-order reach of Walker Branch.

	10 April 1992		29 July 1992		16 November 1992	
Parameter	Original	Revised	Original	Revised	Original	Revised
GPP						
g O_2·reach^{-1}·day^{-1}	140.97	279.42	8.64	23.34	9.72	31.40
μg O_2·m^{-2}·s^{-1}	20.17	40.76	1.44	3.90	1.62	5.24
Percent increase		198		270		323
CR_{24}						
g O_2/reach per day	−235.95	−641.06	−343.73	−600.63	−147.47	−977.88
μg O_2·m^{-2}·s^{-1}	−19.69	−93.53	−28.68	−50.11	−12.30	−81.58
Percent increase		272		175		663

Note: The original values are those reported in Marzolf et al. (1994) and the revised values utilized the reaeration equation suggested by Young and Huryn (1998).

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equation (Table 1). The revised GPP and CR_{24} values are considerably higher than the original estimates (264% average increase in GPP, 370% average increase in CR_{24}) because of much higher reaeration fluxes of oxygen, (223% average increase). These revised CR_{24} values also show a seasonal pattern of substantially greater CR_{24} following leaf fall than at other times.

Young and Huryn (1998) also point out a second, but relatively minor, error in fig. 2 of our original paper. The shaded region depicting daily community respiration CR_{24} should extend to the value of 0 on the *y*-axis. Our calculations for CR_{24} did indeed integrate over the entire region below $y = 0$.

We appreciate the insightful comments by Young and Huryn. They present a more accurate method for computing reaeration flux in the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism. We are sorry for the error that appeared in our original paper. Nonetheless, we believe that the upstream–downstream oxygen mass balance approach is of great value in determining rates of stream metabolism at the whole ecosystem scale, provided that the formulation for the calculation of the reaeration flux suggested by Young and Huryn (1998) above is used.

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CCFFR Web-Site:
http://www.phys.ocean.dal.ca/ccffr/index.html

Canadian Conference for Fisheries Research (CCFFR)

Edmonton, Alberta
8–10 January, 1999

The major themes of the **1999 Conference** will be:

(1) Effects of land use on stream fish and their habitat

(2) Impacts of endocrine disruptors and contaminants

(3) Changes in climate and ecosystems

(4) Innovative applications of genetics in fishery management

(5) **Joint CCFFR/SCL Session**: (to be announced)

Papers addressing the major themes are encouraged, although submissions on other themes related to fisheries and aquatic habitat biology are also welcome.

The **DEADLINE for abstracts is 11 September 1998**. Submit abstracts to the Program Committee Chair:

Dr. Dick Beamish
Pacific Biological Station
Nanaimo, BC V9R 5K6
Tel.: 250-756-7029
Fax: 250-756-7053
e-mail: beamishr@dfo-mpo.gc.ca

Instructions overleaf

For general information contact the CCFFR Secretary Treasurer:

Dr. Howard Powles
Tel.: 613-990-0285
Fax: 613-954-0807
e-mail: powlesh@dfo-mpo.gc.ca

Conference Canadienne de la recherche sur les peches (CCRP)

Edmonton (Alberta)
les 8–10 janvier 1999

Les principaux thèmes abordés lors de la **Conférence de 1999** seront :

(1) Les impacts de l'utilisation des terres sur les poissons de rivière et leurs habitats

(2) Les impacts des disrupteurs endocrinaires et des contaminants

(3) Changements climatiques et écosystémiques

(4) Applications innovatrices des études génétiques dans la gestion des pêches

(5) **Session Conjointe CCRP/SCL** : (sera annoncée ultérieurement)

Des contributions reliées aux thèmes principaux sont particulièrement sollicitées, mais des contributions sur d'autres thèmes reliés aux sciences halieutiques et à la biologie des habitats aquatiques sont également bienvenues.

La **DATE LIMITE pour la soumission des résumés est le 11 septembre 1998**. Veuillez les soumettre au :

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Téléphone : 613-990-0285
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Abstract preparation

(Deadline: September 11, 1998)
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Follow the guidelines below and send an ASCII (text) version of your abstract (do NOT use Word, WordPerfect, or other word processing formats) by e-mail to:

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(1) Place an asterisk (*) after the name of the person presenting the paper.

(2) Indicate whether the presentation will be oral (O; only standard 5 × 5 cm slide projector and overhead projector will be provided free of charge) or poster (P) after the title.

(3) Two concurrent paper sessions will be held and the number of oral presentations will be limited. Please indicate, on a separate line after the end of your abstract, if your presentation COULD be a poster even if your preference is for oral presentation. Please also indicate on this line to which theme (1–5) your presentation is most closely related. This line will not appear in the program.

(4) Follow this format:

Relationships between sunspots and sea stars (O)
Gretzky*, W., and J. Villeneuve. Department of Epidemiology, Eastern University, Yoho, Saskatchewan

Sea stars shine increasingly brightly as the sunspot cycle progresses...

Could be poster; theme 3

Hard copy of Abstract
As CCFFR moves to an electronic process for developing the program, hard copy abstracts will not be accepted except under exceptional circumstances. Contact the Program Chair.

Préparation des résumés

(Date limite : le 11 septembre 1998)
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Copie du résumé envoyée par courrier électronique

Suivre les lignes directrices et envoyer une version de votre résumé en ASCII (texte)(ne PAS utiliser formats Word, WordPerfect, ou autres formats de traitement de textes) par courrier électronique à :

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(1) Placer un astérisque (*) à coté du nom de la personne qui fera la présentation

(2) Indiquer après le titre si la présentation sera orale (O; seuls les projecteurs à diapositives 5 × 5 cm et les rétroprojecteurs seront fournis sans frais) ou affiche (A).

(3) Il y aura deux sessions concurrentes et le nombre de présentations orales sera restreint. Veuillez indiquer sur une ligne à part après la fin de votre résumé si votre présentation POURRAIT être par affiche même si vous préférez une présentation orale. Indiquer également sur cette ligne à quel thème votre contribution est le plus reliée (1–5). Cette ligne n'apparaîtra pas dans le programme.

(4) Format :

Predation par les euphausiaces sur les bebes phoques (O)
Villeneuve*, J., et W. Gretzky. Departement de planctonologie, Université de Trois Pistoles, Trois Pistoles (Québec)

Une augmentation subite de la mortalité des bébés phoques s'est avérée...

Pourrait être affiche; thème 3

Copie du résumé sur papier
Puisque la CCRP est en train de développer un processus entièrement électronique pour la préparation du programme, les résumés sur papier ne seront acceptés que pour des raisons exceptionelles. Veuillez prendre contact avec le Président du Comité du programme.

Asit Mazumder and Karl E. Havens	**1652–1662**	Nutrient–chlorophyll–Secchi relationships under contrasting grazer communities of temperate versus subtropical lakes
M.G. Meekan, J.J. Dodson, S.P. Good, and D.A.J. Ryan	**1663–1673**	Otolith and fish size relationships, measurement error, and size-selective mortality during the early life of Atlantic salmon (*Salmo salar*)
M.G. Meekan, D.A.J. Ryan, J.J. Dodson, and S.P. Good	**1674–1681**	Does mortality select size or growth potential in young sockeye salmon (*Oncorhynchus nerka*)? A simulation based on otolith – fish size relationships
John R. Zeldis, Rob C. Murdoch, Patrick L. Cordue, and Mike J. Page	**1682–1694**	Distribution of hoki (*Macruronus novaezelandiae*) eggs, larvae, and adults off Westland, New Zealand, and the design of an egg production survey to estimate hoki biomass
Frederick S. Scharf, Jeffrey A. Buckel, Francis Juanes, and David O. Conover	**1695–1703**	Predation by juvenile piscivorous bluefish (*Pomatomus saltatrix*): the influence of prey to predator size ratio and prey type on predator capture success and prey profitability
Robin Mahon, Stephen K. Brown, Kees C.T. Zwanenburg, D. Bruce Atkinson, Kenneth R. Buja, Larry Claflin, Geoffrey D. Howell, Mark E. Monaco, Robert N. O'Boyle, and Michael Sinclair	**1704–1738**	Assemblages and biogeography of demersal fishes of the east coast of North America
Dany Pigeon, Julian J. Dodson, and Louis Bernatchez	**1739–1747**	A mtDNA analysis of spatiotemporal distribution of two sympatric larval populations of rainbow smelt (*Osmerus mordax*) in the St. Lawrence River estuary, Quebec, Canada
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